BIOCHEMICAL STUDIES ON BLOOD/SERUM OF DIFFERENT MAMMALS

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By

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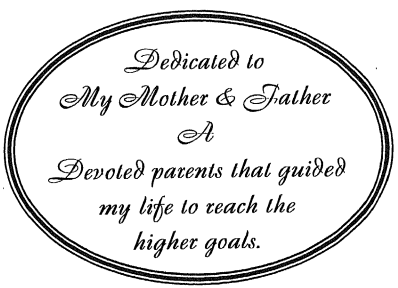
Dedicated to
My Mother & Father
A
Devoted parents that guided
my life to reach the
higher goals.

DEPARTMENT
OF
BIO CHEMISTRY,

FACULTY
OF
SCIENCE

UNIVERSITY OF ALLAHABAD,
ALLAHABAD

CERTIFICATE

This is to certify that matter embodied in this thesis entitled. "Biochemical Studies on Blood/Serum of Different Mammals" by ***Mohammad Ishrat*** *is a record of bonafide research work carried out by him under my supervision and guidance. He has completed all the requirements for submitting this thesis for the degree of Doctor of Philosophy to the University of Allahabad.*

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Introdcution

INTRODUCTION

The aim of the present investigation about which no information is available in comparative "Biochemical Studies on Blood / Serum of different mammals.

The attention devoted by the scientist in this field for the last few decades shows the importance of investigation on comparative studies.

Osmotic fragility of erythrocytes differ between species of animals (Park et. al 1964) and even in the same animal species (Schalm et. al 1975) and this have made it difficult to establish reliable normal values for diagnostic proposes.

The different value may be due to difference in factors that effect the osmotic fragility of erythrocytes, such as age and sex of animals (Part et. al 1964), Sarkar et. al 1998). Temperature and pH of the hytotonic solution (Sarkar et. al 1998 b) and size and valume of erythrocytes (Schalm et. al 1975).

There were wide variation observed in the osmotic fragility (OF) profile and mean erythrocytes pagility (MEF) of different vertebrate species (Rizvi S.I., et. al 1998).

The osmotic behaviour of erythrocyte may be more clearly under stood if the factor's affecting it could be studies in the various mammalian species.

The blood has very important role in metabolism specially erythrocyte metabolsm in man and other animals shows great

variation, particularly with respect to oxygen affinity and the interaction of haemoglobin with 2,3 diphaspho glycerate.

Many of the human complex congenital and hereditary forms of hematological abnormalities e.g. thalassèmia, hereditary ellipto cytosis ànd G-6 PD deficiency have not yet been recognized in lower animals.

All these example emphasize the importance of comparative approach to hematological interpretions.

Blood is a viscous fluid possessing a complex composition.

It owes its viscosity due to the presence of various protein and other cellùlar materials.

Normal viscosity of blodd is found in the slightly alkaline range that is 7.35 - 7.45.

The specificgravity of blood vovis in the range 1.03 - 1.075.

Arterial blood appear to be bright crimson where as kvenous blood appears as dark red.

In multicellular organism the individual tissue cells remain in co-ordination with the external environment and with the other body importance of blood may be easily under stood on the basis of its function in the body.

Blòod act as the principal transport medium for biological substances diverse nature.

The observed dietary materials is transported from digestive tract to all the body tissue.

The excretory products are transported from different tissue to their excretory chennels.

Hormones are transported from the endocrine glands to their target organs. Oxygen is transported from the lungs to all the tissue and carbondioxide from the tissue to the lungs.

Blood also help in the regulation of body water, temperature and acid base balance.

It also provides defence to the body against various types of infection due to the presence in it of antibodies (immunoglobulins) and white blood cells.

A normal adult human individual contains approximately 5 - 6 litres blood in the body which constitutes about 5 - 8 persent of the body weight.

This valume is maintained within narrow limits under normal conditions. Under pathological condition, when blood valume is remarkebly changed serious consequences are encountered and it might some time above to the fatal.

If exuded blood is kept for some times, it coagulates forming a thick clot and leaves a clear strow coloured fluid which is knwons as serum.

Serum remains devoid of Prothombin and contain reduced concentration of some of the coagulation factor as compared to plasma.

The clot is mainly composed of fibrin stands to which platelets red blood cells and white blood cells are closely adhered.

The proteins of blood are mainly haemoglobin alumin, goblins, fibrinogen and some of the enzymes of blood coagulation.

Some non functional enzymes are drained into blood by destruction of older cells of the body.

The non-protein nitrogen fraction of blood includes urea, uric acid, creatinine, creatine ammonia and amio acids.

Among the lipids blood contain free fatty acids, etherified fatty acid, including fats and phaspholipids and free and esterified cholesterol.

The most important protein of the red cells is haemoglobin this protein finds an important place among the other proteins due to its ability to combine with oxygen reversibly. It carries oxygen from the lungs to the tissues and CO_2 from tissues to lungs. Normally haemoglobin is solely confined to the red blood cells. It is a conjugated protein, possessing heme as its prosthetic group and globin as its protein portion.

The heme part of most of haemoglobin is similar but globin portion shows considerable variation in the amino acid composition and sequence. Each molecule of haemoglobin contain four molecules of heme attached to one molecules of globin. The chemical properties of haemoglobin as follows.

1. **Combination with oxygen** - Reduced haemoglobin can loosly combine with oxygen forming oxyhaemoglobin. The attachment with oxygen occurs at iron in the heme portion. Iron is present in the ferrous form both in haemoglobin and oxyhaemoglobin, oxygen remains attached with the unpaired electron of Iron coach heme combined only one molecules of oxygen.

Since each molecule of haemoglobin contains four molecules of heme, hence one molecule of haemoglobin can maximally combined with four molecules of O_2.

The combination of haemoglobin with oxygen is governed by partial pressure of oxygen (PO_2) and partial pressure CO_2 (PCO_2).

The former favours combinxation whereas the later favours dissociation.

Reduced Haemoglobin + $4O_2$ = Oxyhaemo globin (Hb. $4O_2$)

Combination with carbon dioxide :

Haemoglobin does also combine with CO_2 through its amino groups present on the globin portion forming carbomino haemoglobe compound. Formation of this complex is a reversible process.

$Hb\ NH_2 + CO_2 == Hb\ NH.\ COOH == Hb\ NH\ COO^- + H^+$

Normally about 10% of the total CO2 is carried by the blood in this form. Haemogloin can be combine with hydorgen cyanide or other cyanides.

Normal values of biochemical constittents of blood and serum in mammals are of academic as well as clinical importance. Extensive research conducted in India, revealed that, these value vary from region to region (Pandiya et. al., 1977). It is therefore necessary to establish the normal levels of biochemical constituents of blood and serum in mammals of particular region.

The mammals has to be maintained in perfect state of health. Any deviation in health proportionately alters the production. To assess whether the mammals is in a perfect health state or not, the bio chemical analysis of blood sample is frequently employed.

Accordingly the metabolic profiles lest appears a good diagnostic tool for the early diagnosis and prediction of the production diseases. The tests need normal values of blood parameters from the population within the area.

The effect of short term water deprivation have been reported in Marwari sheep under arid condition (Taneja, 1996) and in Beetal and Black Bangal goats (Kauhish et. al, 1993) and lambs under semi-arid condition (More and Sahni, 1978). The effects of elements on good wire reviewed by Jindal (1980). However, the information on haematological responses on the effect of heat and water deprivation in mammals is limited in our country, hence this study.

The present study was undertaken to determine the effect of age on levels of blood/Serum biochemical constituents in different mammals.

Knowledge of the variation in serum of plasma concentrations of biological indicators during a 24 - hrs. period is important for a better understanding of circadian cycle effects on metabolism. It can also enable an evaluation of the best time to collect blood in order to be able to correctly interpret metabolism mechanisms or status. In mammals fed a single daily meal, the variation of those indicators during the 24 hrs. period can also be seen as a simulation of the metabolic response to successive phases of food availability and deprivation.

Modern blood analysis technologies allow easy, inexpensive and simple assessment of mammals metabolic status, allowing the evaluation of the suitability of feeding plans for the expected levels production.

Several blood variables have been utilised as indicators of physiological status (Blowey et. al) 1973 ; Lee et. al 1978 ; Payne and Payne 1987). The purpose of this trial was to evaluate the 24 - h

variation in some of these blood variables and to determine the most suitable time for blood collection to maximise the diagnostic value of these indicators in mammals exposed to different levels of feeding in a single daily meal.

The nutritive value of a feed protein depends primarily on its capacity to satisfy the needs of mammals for amino acids. Different protein sources may have different nutritive values depending on their ability to provide essential amino acids for the various biochemical functions of the organism. As the essential amino aid requirements may vary according to the age and physiological status of the mammals (maintenance, growth, pregnancy, lactation, etc.) and the type of production (meat, milk, egg, etc.), the same protein consequently may have different nutritive values, depending on the criteria used for its evaluation.

The protein quality of dietary proteins is primarily determined by the content and proportion of their available amino acids. However, the nutritive value of a feed or feed ingredient is not independent of the mammals to which it is fed. The composition and adequacy of the diet as a whole and the physiological, nutritional and health status of the mammals influence the capacity of the feed protein to meet the amino acid requirement of the mammals.

Levels of dietary protein, energy, fibre, tannin, the so-called ant nutritive substances as well as environmental circumstances (housing, temperature, light programme, etc.) and the feeding techniques all may influence protein utilization to a different extent. Species, strain, age, sex, health and physiological status also may have an effect on protein digestion and absorption. In bioassays for evaluation of the protein quality these factors are to be held more on less constant to get reliable results depending primarily on the digestibility and amino acids composition of the raw protein tested.

This gives a short overview of the most important factors which are independent of amino acid composition of the feed protein and may influence protein utilization.

Most mammals are known to utilize fat, the nutrient richest in energy, very well. On the other hand, the digestive physiological characteristics of herbivorous animals, particularly ruminants, restrict the utilization of that nutrient. Fat is not a natural feed for ruminants.

Although fats are efficiently absorbed from the intestine of ruminants (Andrews and Lewis, 1970 Steele and Moore, 1968) their presence in the rumen may impair the efficiency of fermentation. The digestibility of crude fibre decreases as natural fats "enwrap" (coat) the feed particles present in the rumen fluid and make them hardly accessible to bacteria (Rohr et al., 1978).

Animals were taken out for grazing under supervision of a grazing at 8 hr and allowed free grazing till 17hr and herded in aside open animal shed till next day. In addition to free grazing

Grazing animals generally do not receive mineral supplementation, except for common salt, and depend almost exclusively upon forages for their nutritional requirements. However, tropical forages can rarely satisfy all mineral requirement (Pfander,1971). Thus sub-opumum nutrition is often nutrition is often accepted as the most important limitation to liver stock, production in tropical countries (Mc Dowell et. al., 1983). Nutritional anaemia or salt sick is common finding which is reasonably causing poor livestock production. There was no report on blood profile of cattle maintained solely on grazing. The objective of this study was to report the spontaneous cases of anaemia in grazing cattle.

The present experiment is an attempt out the effect of starvation (24 Hrs.) in different mammals also shows great variation in heamatological profile.

The study of serum cholesterol has attracted much attention in recent years. Cholesterol is an important constituent of blood being the precursor of several hormones responsible for production and reproduction in mammals. Serum cholesterol is the key precursor of optimum equilibrium in endocrine inter-relationship. Several reports from the works of scientists indicate a definite relationship between blood cholesterol and productive traits in animals. The classical work of Wilcox and Shaffner (1963) on poultry and Mi et. al. (973) on pigs are case in point.

The liver is the key organ in the metabolism of cholesterol in humans. It is the only organ by which substantial amounts of cholesterol are excreted from the body either directly as free cholesterol into the bile or after conversion to bile acids :

(1) The liver plays important role in serum lipoprotein synthesis and metabolism. Hence liver malfuncton is expected to produce changes in serum lipid and lipoportein levels

(2) Free cholesterol is in rapid equilibrium between serum lipoproteins and red cells. The level of red cell cholesterol is influenced by bile salts and bilirubin (3), which shifts the serum / cell partition of free cholesterol to cell phase and which inhibit the cholesterol esterifying mechanism. Any process or disorder affecting cholesterol exchange in vivo is capable of critically modifying the shape and behaviour of red cells which result increased resistance to osmotic lysis

Enzymes : Enzymes are a very important group of proteins which catalyse practically all chemical reactions in biologic systems. They are reactions are enzyme catalysts. Since essentially all biochemical reactions are enzyme catalysed many different enzymes exist in the organs of the body. They are intracellular and hence in health only small amounts are present in plasma/Serum. But when some disease process leads to increased cell breakdown in an organ, enzymes may escape in greater quantity with consequent increase in plasma. Therefore measurement of serum enzyme activity provides an important diagnostic tool for recognition of disease and organs involved. The extent of the rise in serum activity of these enzymes involved. The extent of the rise in serum activity of these enzymes depends not only on the concentration of the enzyme in the tissue and the extent to which the organ is damaged but the organ is damaged but also on (i) the rate at which the enzyme escapes from the damaged tissue (ii) changes in permeability of cell membranes and (iii) the rate at which the enzyme is removed from the plasma either by metabolism or by renal excretion.

An enzyme transforms a compound (substrate) into another compound and the rate of transformation depends on the enzymatic activity. So the estimation of enzymes differs from other biochemical tests in that they involve measurement of rate of reaction rather than the quantity. Various factors such as temperature, time, pH, presence of inhibitors and activators influence the enzymatic activity and therefore these conditions must be carefully controlled in its measurement. Enzymes are proteins and they must be protected from denaturation. The serum should be kept at 4^0C or frozen if analysis is delayed. Repeated freezing and thawing should be avoided.

Isoenzymes - Different forms of an enzyme having similar enzymatic activity are called isoenzymes. Isoenzymes have very similar chemical and physical properties and catalyse the same reaction but originate from different sources and have different moleclar weight and mobilities on electrophoresis. They have different relative activities towards different substrates and being differently depressed by inhibitors. In recent years several isoenzymes of some enzymes of clinical importance have been identified and found useful in the differential diagnosis and also in recognizing the organs involved in the disease.

Alkaline phosphatase (ALP : EC 3.1.3.1) has long been studied in many organisms. The wide distribution of ALPs in nature shows that these enzymes are included in fundamental biochemical processes. The strong activity in the intestine, kidney, mammary gland and placenta suggests that ALPs are connected with transport mechanisms (McComb. et. al., 1979).

Generally, these ALP isozymes in mammals are known to be membrane-bound proteins anchored by a phosphatidyl insoitol glycan. (low et. al., 1986 ; Howard et.al., 1987 ; Fishman, 1990 ; Moss, 1992 ; Bublitz et. al., 1993). In relation to the elevation and conversion of serum ALPs in some diseases, the releasing mechanism of membrane-bound ALPs has been discussed (Moss, 1984b; Hawrylak and Stinson, 1990 1988 ; Hamilton et. al., 1989; Fishman, 1990 ; Moss, 1992, Stigbrand and Wahren 1992).

ALPs in a variety of organisms indicate common properties in hydrolase/ transferees reactions, dimeric structure, activity dependence on Zn^{2+} and Mg^{2+} and identical amino acid sequences around the active site. On the other hand, human ALP shows a differentiation into isozymes with an organs-specific distribution, each organ has a distinct properly controlled by a distinct gene (Mc

Comb et. al., 1979); Sugbrand.1984a.b ; Harries, 1989, Millan, 1990; Moss, 1992) Comparative studies on the ALP proteins and DNAs from human and other mammalian tissues have been carried out (Goldstein et. al., 1982, Beckman and Beckman, 1984 ; Doellgast, 1984; Beckman and Beckman, 1984 ; Doellgast, 1984; Fishman, 1990 ; Manes et. al., 1990 ; 1984; Fishman, 1990; Manes et. al., 1990; Beckman et. al., 1992), but more extensive investigations involving various mammals are necessary.

Insects are thought to have evolved specifically and to include divergent species, but relatively little information is available about insect ALPs, ALP isozymes of the silkworm, Bombyx mori represent specificities in some properties such as monomeric structure, existence of a soluble form and uniquencess in amino acid inhibition (Eguchi, 1975; Eguchi et. al, 1990). These characteristics of the insect ALPs will afford valuable informationas to the physiological roles of ALPs. To elucidate the properties of these ALP isozymes, we invistigated, from various view-points, biochemical characterization, immunological studies, enzyme histochemistry, genetics and molecular biology. A significant number of isozymes are considered to share in enzymolgical functions. ALP isozymes in various organisms from this view-point seem to be less elaborated.

Subsequently, considering unique features of the ALPs, properties of the mammalian ALPs are compared. Although the biological, I would like to possible activity.

Lactate Dehydrogenase (EC-1.1.1.2,7) :

Lactate dehydrogenase is widely distributed in uman tissues but found especially plentiful in cardiac and skeltal musceles, liver, kedney and red cells. It catalyses the reaction of transformation of pyruvic acid to lactic acid, and lactic acid to pyruvic acid.

Increased activity is observed in liver disease, particularly in infective hepatitis but the increase is not so great as that of SGOT and SGPT. Normal values are often found in cirrhosis and post hepatic jaundice. The diagnostic significance of LDH is particularly in heart and liver involvement, but the estimation of SGOT and SGPT are simpler and less expensive and have been found more sensitive and rather specific tests for these diseases than LDH. Thus assay of LDH does not show any particular advantage over the transaminases, unless isoenzymes are determined which is complicated and not possible in routine analysis.

Amino transferases (Transaminases) :

The enzymes which are involved in transfer of amino group (CH_2 - NH_2) from an alpha amino acid to an alpha keto acid are called transaminaases and the process is called transamination. Transmination reaction is an important step in the metabolism of amino acids. Two enzymes occur in human tissues which catalyse reactions of this type : aspartate amino transferase popularly known as GOT (glutamic-oxaloacetic transaminase) and alanine amino transferase, GPT (glutamic-pyruvic transaminase). Both transaminases are widely distributed in human tissues, GOT activity almost always being greater than GPT activity.

Pregnancy, parturition and location being physiological stress conditions, the adaptive physiological stress conditions, the adaptive mechanisms are put into work so as to maintain the physiological balance during peri-parturient stages. The effects of these efforts made by various systems of the body are ultimately reflected biochemically.

Serum exzymes activity undergo changes during growth pregnancy and lactation. The information of enzymes during

pregnancy in mammals is scanty and hence the present investigation was carried out in different mammals.

The present thesis work has been carried out on blood / serum of mammalian species like human (homosapiense), goat (Capra Hircus), sheep (ovis-ovis) Cow (Bos indicus) and Dog (Canis familiaris). In order to understand the various studies described in this thesis, it is necessary to have some idea about blood / serum of different mammals.

* * * * * * *

Review of Literature

REVIEW OF LITERATURE

2.1 Blood : Blood may be described as a specialised connective tissue (vital fluid) in which there is liquid intercellular substance known as plasma and formed elements, the red blood cells, the white blood cells and the platelets suspended in the plasma. The specific gravity of whole blood vovis from 1.055 to 1.060. When freshly shed, blood (vovis) from 1.055 to 1.060 slightly alkaline. In order to understand the full significance of blood, it is imperative that one should known something about its genesis.

The birth of the higher forms, the open system become a closed one. This change represents a very important landmark in the history of animal evolution. The sea water, which used to remain outside the body, became entrapped within the body. In the course of evolution, that enclosed marine water has undergone profound modifications and has been transformed into what we call now blood. Although blood has departed a long way from its primitive ancestor, yet in its inorganic composition, it still maintains a close resemblance with sea water. This, in short, is the history of evolution of blood.

In the multicellular animals, blood serves those purposes, which, for the unicellular forms, are carried out by the surrounding aquatic medium. But in addition to those, blood has to serve many other important functions, because it has to meet the complex requirements of the more specialised cells, in the higher forms.

Blood accounts for 3- 5% of animal live weight (Terrell, 1961). Composition of blood and plasma ovis from individual to individual as ell as from day to day samples (Swenson, 1984). Whole blood

contains about 80% moisture, 18/5 protein, 2% inorganic materials and a negligible amount (0.1%) of fat (Hald-Christensen, 1986 ; Stiebing and Wirth, 1986b; Flistrup, 1987).

Plasma contains about 6-8% protein of which albumin is 505, globulin 23-27% and fibrinogen 17-23%. Total protein content of there cells is about 28-30%, the major component of which is globin (Ledward and Lawrie, 1984 ; Swenson, 1984).

Chemical composition of blood protein isolates depends on several factors involved in their preparation. These are method of stabilization, coagulation, separation, preservation, processing (chemical or thermal) and drying (Delaney, 1975 ; Tybor et. al., 1975 Anon; 1978 ; Penteado, 1979 ; Quaglia and Massaci, 1982 ; Stiebing and Wirth, 1986 b and Piot et al., 1988).

The proximate composition of plasma protein isolates was determined by many workers (Delaney, 1975 ; Khan et. al., 1979 ; Del-rio-de-rays et. al., 1980). They reported significantly higher protein content and low as content. The dialysed plasma samples also gave similar results (Delrio de reys et. al., 1980). Quaglia and Massacci (1982) observed that use of proteolytic enzymes in place of anticoagulants gave high quality protein with low level of ash. To minimize the denaturation of heat sensitive proteins concentrating and drying treatments were advised by them.

Chemical composition of globin protein isolate has been studied by various workers (Taybor et. al., 1975 ; Berezhnaya, 1984 ; Sato et. al., 1981 ; Hayakawa et. al., 1982 ; Olsen, 1983 and Abo Bakor et. al., 1986).

Tybor et. al. (1975) reported that the globin and plasma protein isolates contained 91% and 71% protein respectively. Shahidi et. al, (1984) recorded 81.3% protein with high salt content in boving globin protein isolate. Kormendy et. al. (1987) also reported the presence of moisture 5.0% protein 80% and ash 15% in globin.

It contain about 18-19% protein like lean meat and is sometimes referred to as "liquid meat" (Wismer Pedersev, 1979) Protein content of plasma is 7-8% while, red blood cells (RBC) contain 34 - 38% protein. Being rich in protein and other nutrients blood is having the highest pollution capacity. The meat industry is under constant pressure to improve the utilization of slauter house by products especially blood.

2.2 Use of Anticoagulants :

To maintain the liquidity of blood, use of anticoagulant is required. Anticoagulants on the chemicals or agents which prevents clalting of blood by inhibiting any one or more of the factors involved in clotting mechanism.

Most common anticoagulatn is sodium citrate and is used at the level of 0.2 - 0.68% (Moorjani et. al., 1978). Papain and other proteolytic enzymes (fibrinolytic) can also be used as anticogulant agents (ouaglia and massacci, 1982). Use of phosphate as anticoogulant has been reported by Gordon (1971) and use of di-sodium salt of etylee diamine tetra acetic acid (EDTA) has been patented by hipnear (1969).

Stiebing and Wirth (1986 b) ad Bijoric et. al. (1993) studied the effect of sodium citrate on the quality of plasma. A reduced protein content and increased plasma yield were observed with the

increased levels of citrate. Matekalo et. al (1993 a) Studied the effect of anticoagulants at 1% level on hygienic quality of bonine blood. Use of all the three anticoagulants (Sodium citrate, sodium phosphate sodium pyuno phosphate) result in higher bacterial count and shorter shelf - lief conpared to control samples. Matekalo et. al (1993 b) obsrved that sodium lactate along with citrate or phosphate reduced the bacterial count. Yield of plasma increased and colour also improved by the addition of lactate.

Farnandot (1976) adopted amechanical method to prevent cogulation, by whipping the blood immediately after collection with a five beater. However, partial haemohysis of RBC resulted in redish tiged plasma.

2.3 Separation of Blood :

Blood should be separated into plasma and red cells within 20 min of collection (Halliday, 1973). Separation is accomplished by centrifugal force. Degree of separation depends on shape of the rotor and speed of ration. Plasma is separated from red cells at about 1400 rpm on continuos or 5800 rpm for 10 min on non-continuous basis. There are several type of equipment's capable of processing upto 5000 litres of blood per hour (Akers, 1973). Ultrafiltration has also been used for the separation of blood with little haemaolysis. (Porter and Michaelis, 1971 ; Grigoror et. al. 1991).

Simple portable milk separator can be used for separation of blood into plasma and RBC concentrate. (Tybor et. al., 1973 ; Rareltzis and Buck, 1985).

Autio et. al. (1984) used Alfa - haral centrifugal separator. Delaney and Donelly (1974) and Delaney (1975) separated blood on

wesphalia centrifugal seperator at 6300 rpm and flow rate of 12 litres / mim. Penteado et. al (1979) stroed blood at 4°C and seperated within 36 hrs of collection by decantation followed by centrifiguation. Caldironi and Ockerman (1982) Stored blood at 4°C for 24 hrs. and seperated by centrifugation at 3000 rpm.

Separation of blood yields about 70% plasma and 30% red cells (Delaney, 1977). There are variable reports on the yield of plasma. Gracy (1986) reported the recovery of 60-70% plasma and 30 - 40% red cells. Filstrip (1987) also reported similar results. Pre Chilling of blood y ore separation leads to higher plasma yields (Akers, 1973)

Haemolysis occur to some extent on physical disruption of red cells and plasma is tinted reddish in colour. Haemolysis occur also with the addition of hypotonic solution, sudden change of temperature and rough handling (Gracy - 1986).

2.4 Microbiological Quality of Blood :

Microganisms which spail blood mainly comes from intestine, stomachs, gutfill, hide, equipments and handless etc. (Tybor et. al., 1975 ;. Swingler, 1982) Heinz (1969) and Borowski (1981) emphasized on the cleaning and streilization of equipments and washing of animals before bleeding.

Fresh blood from healthy animals is free from bacteria (Haines, 1937) ; Richard, 1970). When aseptically collected initial microbial count should be 200-300 C.F.U. and 2000-3000 C.F.U. per ml for beef and pork blood respectively (Nilsson, 1975). Type of bacterial is more important than the number of bacteria (Knipe, 1988). Gunstone (1980) and Walkowiak (1980) observed that fresh blood

never contained clostridium or Salmonella. However, thye invariably found Enterobacteriaccae, Protcus and Escherichia. Staphylococcus accreus was ocasionally found but mostly were cogulase positive (non-pathogenic). Total bacterial counts ranged between 1.3×10^5 - 1.8×10^5 in whole blood and plasma of pig and cow.

Anon (1983) reported aerobic mesophilic count to be 4×10^4/ml at 32°C . However Chen et. al. (184) reported total plate count of 1.8×10^6 - 2.26×10^6 / ml in refrigerated RBC concentrate.

2.5 Preservation of Blood :

Because of high water and protein contents and high pH values, blood tends to spoil very easily and quickly. For this reason, immediate preservation of blood is essential (Stiebing and Wirth, 1986 b). Refrigeration has been found to be one of the best methods for preservation of blood (Bachstein, 1942).

Breer (1978) reported that plasma could be stored frozen with excellent bacteriaological quality. No quality changes were observed during storage of plasma at - 25°C upto 15 weeks (Nakanura et. al., 1983). Lerche (1939) preserved plasma by freezing for several months without deterioration in quality but cautioned that it should be used immediately after throwing.

Chemical preservations are also emploed for the preservation of blood or blood plasma. Zimmerman (1982) stored blood plasma for 3 days at ambient temperature by adding 3% sodium chloride. Phosphated plasma has been storage life than citrate plasma (Kotter and Terplan, 1960 ; Heinz, 1969) Acid citrate dextraose (ACD) and citrate phosphate dextrose (CPD) have been used as preservative cum anticogulants (Masceuredis, 1983).

A poly phosphate ammonia mixture has also been shown to preserve the blood upto 18 hrs without refrigeration (Akers, 1973). Blood has bee stored for 15 days at 5° - 6°C sodium chloride (10%) and 14 days at room temperature with liquid ammonia (0.1 : 1-0, 2%) by Uchman et. al. (1980).

2.6 Function of Blood / Red Blood Cells :

The primary functions of the red blood cells are the transport of oxygen to the tissues and carbon dioxide to the lungs. In order to carry out these activities the developing red cell synthesize the many millions of haemoglobin molecules the oxygen carrying pigment of the blood. In the addition to haemoglobin the erythrocyte contains numerous complex enzyme systems, some necessary for efficient respiratory function and some essential to its own metabolic need. Normal matured red cells, survive in the circulation for approximately 110-120 days. In order to maintain the number of red cells in the blood new cells must regularly be formed by the bone marrow. If its life span is significantly shortened, the red cell mass falls and relatively less oxygen is transported to the kidneys and hence there is an increased output of erythropoietin which results in an increased rate of erythropoiesis. The blood reticulocytes develop as erythrocytes over a period of one or two days. The red cell mass can be restored to normal in this way. Like other animal cells the erythrocyte is surrounded by a cell membrane. The haemoglobin enclosed within the erythrocyte membrane perform the principal functions of the red cell. Haemoglobin are large, complex protein molecules comprised of four polypeptide, each containing one molecules of heme. It has the ability to bind oxygen efficiently in the lungs and to release a large proportion of it at some what lower oxygen tension which is found in the tissues and is essential for the

survival of large oxygen requiring organisms. The principle allosteric factors modulate the oxygen delivering properties of hoaemoglobin are hydrogen ions and 2, 3 - diphasphoglycerate (2, 3, DPG). When tissues are deprived of oxygen their pH falls and decrease in pH decreases the oxygen affinity which facilitates release of oxygen by haemoglobin. The production of 2, 3 - diphosphoglycerate and intermediate which is important in the control of oxygen affinity is produced via a branch of the glycolytic pathway, the Rapoport - Lumbering pathway.

In order to maintain the haemoglobin in a functional state and to keep the red cell readily deformable, energy is required. This is suplied to the red cell by the relatively simple metabolic pathway of glycolysis, the Embden - Meyerhof pathway and the oxidative pathway, the pentose phosphate pathway. Energy is translated in two main ways, either as ATP produced form glycolysis or as reducing power in the form of NADH for the reduction of methemoglobin or NADPH produced from pentose phosphate pathway.

2.7 Red Blood Cells (Eythrocites) :

There are three phases in the life cycle of the red blood cell. The first is a brief period of the order of 5 - 7 days, during which the red blood cell develops in the bone narrow from its precursor stem cell. This is a period of differentiation, haemoglobinization and extrusion of the nucleus. It is followed by the entry into the blood stream as a reticuleecyte and there after a relatively long period (in human approximately 110 - 120 days) in which the red cell circulates in the blood. At the end of this period there is a brief

phase during which the red cell is removed from the circulation and catabolized by the cells of the reticuloendothelial system.

The mammalian mature cruythrocytes (with the exception like camel) are non-nucleated biconcave disc-like structure and they import red colour to the blood because of the haemoglobin which constitutes about 97% of the dry weight of an erythrocyte. In the multicellular organism due to its typical function of carrying oxygen in the form of oxygenated haemoglobin, The red cells have capability to squeeze through capillovis much narrow than their own diameter and reverting rapidly to the original shape after emerging out. This is due to floxible super macro molecular arrangement of bio macromolecules in the termo dynamically favoured state of its membrane.

The shape and the associated mechanical properties as well as the composition of the membrane in respect of the major protein constituents are common to most mammalian red cells. The best documented exception is the camel which has cells of elliptical type containing an exceptionally high proportion of protein and an elevated ratio of integral to peripheval proteins and exhibiting remarkable resistance to osmatic lysis (Eitan et. al 1976) Avain red cell are different again, most notable in that they possess peripheral microtubules. The major peripheral (Cytoskeletion) protein spectrum or at least and equivalent appears to be a feature common to all erythroid cells, even those of an invetebrate (Pinden et. al., 1978).

The read blood cells exist primarilly to transport the respiratory gases. Normal humen red cells at rest are typically in a biconcave disc configuration and are named discocyte, but normal adult cells are deformable & in environment of the circulation are

highly pleamorblic passing through broad spectrum of transitional shapes. In the circulation there is a progressive conversion of the biconcave disc into an ellipsoid, the long axix of which is in line parallel to the direction of flow and the upstream end of which is flattened by the pressure gradient in the blood vessel (Skalak and Branemarks, 1969) Schmid Schonbe in and Wells, 1969).

The red cells of non-mamalian species are usually nucleated and have a relatively complete set of metabolic reactions sequence and a fairly active metabolism. In contrast, the non-nucleated red cells of mammals have only segments of many pathways with some pathways missing and metabolism tends to be sluggish. If appears that evolution has moved towards red cells lowering item metabolic activity, at least as viewed from the stand point of carbohydrate metabolism. Thus the ATP probably the most important product of red cell metabolism, is lowest in the mammals, some what higher amphibians, and highest in fish. It may be that as cells with lower metabolic activity evolved, the need for the nucleus diminished certainly the mature red cell does not require its DNA for further mitosis, and with less metabolic activity, active transcription and translation decrease in importance. Thus the complete loss of nucleus in mature mammalian red cells, may be a part of continuing evolution toward less metabolic activity. The mature human red cell is also incapable of synthesizing protein during its life-span and must "make do" wit the proteins it has previously synthesized.

2.8 LIFE Span of Erythrocytes :

In Human the normal potential red cell life span is of the order of 115-120 days. The data obtained by various methods in human and the order species studied are given by Berlin et. al., (1959)

Indifferent mammals the life span of red cells is about 125 days (Kaneka and Cornelius, 1962). The life span of sheep (Ovis aris) Erythrocytes is about 80 - 135 days (Carter et. al, 1964), whereas in case of cow (Bos indicus) the life span is about 160 days, and in case of dog (Canis familiaris) the life span is about 107-115 days. (from land book of vetemary clinical pathology).

2.9 Erythrocytes in different Species :

Human (Homo sapiense) : The human red cell a flattened biconcare disc lacking a nucleus. The shape is maintained by the submembranous cytoskeleton giving the cell a large surface area than would a spherical shape. This, together with the smaller diffusion distances involved, facilitates gaseous exchange in and out of the cell. (The total surface area of a human's red cells is about 4000 m^2)

Goat (Capra Hircus) : The RBC are smallest among domestic animals. They are round to blunty triangular. Stains uniformly with no central paller area. Variation in size present.

Sheep (Ovis Ovis) Rouleaux formation prominent. Sharp point creation is common. No central paller area.

Cow (Bos Indicus) : The RBC are circular and biconcare discs. Anisocytosis is normal in slight degree. Rouleaux formation is absent.

Dog (Canisfamiliaris) : The cells are uniform in shape with slight variation in size. Present a well defined area. Rouleaux are commonly present.

2.10 Causes of Different Shape & Size of Erythrocytes :

In order to carry out these functions red cells need to be easily deferrable to pass through the capillary network of the body and the structural integrity of the haemoglobin must be maintained. Deferability of red cells is important to its functions in several aspects. Firstly the ability of the red cells to deform permits them to pass through the capillovis of the microcirculation whose dimension are much smaller than those of the resting discocytic cells (Branemark and Bagge, 1977). Secondly, the deformation of flowing cells cause a reduction in the bulk viscocity of blood flowing through large vessels (Chein, 1970). In addition, it may be that a progressive increase in the deformability of the maturing reticulocytes provides a mechanism to allow release of these cells from the marrow into the circulation (Leblond et. al., 1971). Finally it may be that a loss of deferability promotes the removal of non-functional and aged cells by the retculoendo thelial system (Brovelli et. al., 1977 ; Suhail and Ahmad, 1988). So, it appears that the red cell deferability is directly related to its survival. The main factors which determine the cellular deferability are (1) the surface area to volume ratio, (2) the internal viscosity, and (3) the physical properties of the membrane. The properties of the normal red cell represents an optimum adjustment of these factors for maximum deferability. The unique biconcave discoid shape of erythrocytes provides a significant excess of surface area in relation to its volume. The discoid shape of the red cell is determined by the membrane and is a consequence the excess of membrane are a relative to cell volume and of the physical forces in the membrane. Although the red cell is highly deformable and

readily alters shape at constant surface area, the membrane is highly resistant to dilation thereby accounting for the degree of resistance to hypotonic osmotic lysis (Evans, 1973 ; Evans and hacelle, 1975). Since erythrocytes are free from cellular organelles, it is easy to isolate red cell membrane with some exception like camel which has nucleated red cells. Red cell osmotic lysis yields membrane preparations which can be washed so that they are free of haemoglobin.

2.11 Serum :

When blood is shed it loses its fluidity in a few minutes and sets into a semisolid jelly. This phenomena is called coagulation or clotting. On further keeping, the retracts to a smaller volume and presses out a clear straw - coloured fluid, called the serum. Serum will nat clat any more. The clear serum obtained is transfered care fully to another tube for use.

The serum is a clear non-cellular fluid. It contain only serum albumin and serum globulin. Electrophoretic method of separation give a ratio 1.2 : 1.0 this ratio vovis in different species but in the same species it remains almost constant in blood, lymph and serous transudations. In liver disease however due to diminished formation of albumin, the ratio may be reversed. Chemical analysis of the total serum practices reveals that arginine / lysine ratio is 10 : 18. This ratio remains more content than the albumin / globulin ratio.

Although it is customary to state that plasma contains several types of proteins yet it is highly probable that in the living animal all these different varieties remains combined together forming single protein complex. This complex is very loose and is easily broken down into different parts by addition of salts, alteration of pH, etc.

The so-called serum albumin, serum globulin, fibrinogen, etc., are the parts of the same parent complex isolated by different techniques of separation.

Ideally about 50 percent serum is obtained from the blood volume. The serum is kept in a cold place or may be frozen if the analysis is delayed. Serum is similar to plasma in composition but there is no fibrinogen and also a diminished concentration of other clotting factors such as prothrombin :

1. Normal Serum - It is clear and light yellow in appearance.

2. Haemolysed serum - Reddish in colour, haemoloysed serum is not suitable for analysis.

3. Icteric serum - Golden yellow in colour ; found in jaundiced cases due to increased bilirubin.

4. Lipaemic serum - Milky in appearance due to excess lipids in blood. It is also some times found when blood is drawn just after a rich fat diet.

* * * * * *

Material & Method

MATERIALS AND METHODS

3.1. Materials used :

Chemicals :

The following chemicals and biochemical were purchased from the following sources.

3.1.1. - Sigma Chemical Company St. Louis Missouri, U.S.A. :

Albumin bovine Serum

Ethylene glycol-bis (b amino-ethyl ether, N.N.N.N. tetraacetic acid).

3.1.2. Glaxo Laboratories India Ltd. : pH Buffer tablets standards pH - 4.00, pH = 7.00 & pH = 9.2

3.1.3. Loba cheme Indo Autranal Co. Bombay :

- Alanine
- Arseno molybdate
- Ammonium oxalate
- Ammonium Patassium oxalate tablet.
- Barium Hydraoxide
- B -mercepto ethanol.,
- Bromine Water
- Benzoic acid
- B. glycerophasphate
- Cholesterol
- Creatinine
- Citric acid
- Cupper Sulphae
- Disodium ortho Arsenate
- Creatine
- Diacetyl monoxime
- Ferric Chloride
- Sodium β - glycero phasphate
- Thiosemi Carbazide
- α- Keto glutarate
- Sodium pyruvate, 2,4, dinitrophenyl hydrazine DNPH

3.1.4 : B.D.H. Chemical, E. Merck (India) Ltd.

- Dextrose
- Sodium Carbonate
- Sodium Potassium Tarterate
- Trichloro acetic acid (TCA)
- Picric acid.
- Urea
- Zinc Sulphate

3.1.5. Qualigens fine Chemical Mumbai :

- Ammonium molybdate.
- Ethylene diamine tetra acetic acid EDTA

3.1.6. Coral Clinical Systems Goa :

- Heamoglobin Standard.
- Ferri cyanide - reagent

All other chemicals used during the course of this research work were of analytical grade and were of highest purity available. Glass double distilled water were used for the prepartion of reagents and other solution fresh solution and reagents were prepared prior to each experiment.

3.2 Assay Kits Used :

Glueose (Auto span Diaynositc Ltd. Flat No. 336, 338 Surat (India).

Total Protein -- do --

Albumin -- do --

Urea -- do --

Cholestrol -- do --

Alkaline Phasphatase (EC. 3.1.3.1) -- do --

Creatinine (MERCk Limited Worli Mumbai - 400018

Lactate dehydrogenase (E.C. 1.1.1.2.7) Reckon Diagnostics Pvt. Ltd., Gorwa Baroda 390016 (India).

Serum glutamic oxaloacetic transaminase (S.G.O.T.) Bayer Diagnostics India Ltd. Baroda Gujrat (India).

Serum glutamic pyruvic transaminase (SGPT) -- do --

3.3 Washing :

All the glass weres used during experiments were thoroughly washed with detergent (teepol) and chromic acid followed by prolonged drench in tap water and then rensing with glass distilled water.

These glass wares were dried in hot oven. Such washing procedure was followed because it eliminates the possibility of residual detergent contamination which could affect the Blood/Serum sample.

3.4 Measurment of pH :

For measuring the pH of reagents the digital pH meter manufactured by Scientific Instrument Company Ltd. (SICO) Allahabad (India) was used the solution of the standard buffer teblets of pH 4.0, 7.0, and 9.2 manufactured by Glaxo Laboratories (India Ltd. were used for the calibration purpose).

3.5 Optical Measurements :

The colorimetric estimation of protein and albumine were performed by using SICO SPEC - 10 spectrophoto meter (SICO Allahabad India) and Photo Electric Coloricmeter (Model AE-11)' Arma optical work Ltd. Tokyo Japan.

Enzyme assays were performed on Shimadzu double beam spectro photo meter (UV- VIS 150-02).

Autoanlizer- RA-50 chemistry analyzer use for kit

3.6 Enzyme Units - Enzyme activity has been expressed in a variety of ways usually related to the name of worker who developed the analytical procedure, such as Bodensky unit, king Armstrong unit somogyi unit karman unit etc.

The commission of enzymes of the inter national Union of Bio Chemistry recommended that all activities should be expressed in terms of International units (I.U.).

An I.U. is defined as the activity which transforms one micro-mole of the substance in one minute under defined condition of pH and temperature.

The I.U. is expressed as I.U. per litre.

3.7 Blood Donors Mammals :

The Blood from healthy mammals man (Homosapiense), Goat (Capra Hircus), Seep (Ovis-ovis), Cow (Bos indicus), Dog (Canisfamiliaris) were collected by following safe bleeding procedures adapting established techniques special care was taken not excite them prior to bleeding on the basis of history given by owners and special attention was given to avoid the use of any infected or diseased mammals. The mammals were examined clinically and their blood faces and urine sample were analysed routinely to rule out the possibility of concurrent infections.

3.8 Equipment :

Syringe, Needle, Collection Tube, Sprit, Cotton, Etc.

3.9 Sites of Blood Collection :

The blood for examination is collected by venepuncture. The choice of vein to be punctured differs with species. Following are important veins from which the blood can be collected.

Humen - Venous

Goat - Jugular Vein

Sheep - Jugular Vein

Cow - 1. Jugular Vein, 2. Ear Vein

Dog - 1. Cephalic vein (Fore limb), 2. Recurrent Tarsal Vein (Hind limb)

3.10. Distribution of Blood :

- The fluid blood is about 6 - 8% of lean body weight.
- Bone marrow is about 2% of the body weight or roughly equivalent to 1/3 of the valume of liver.

3.11. Techniques of Blood Collection :

3.11.1 Venous : Venous blood is used practically in humen blood examination. It is taken from a prominent vein on the front of the elbow or forearm. The arm is gently swung back and forth which improves the circulation and destends the vien. The arm is extended and a rubber tour required is firmly applied a few inches above the elbow.

The skin over the vein is cleaned with sprit swab and allowed to dry. A well sharpened sterile hypodermic needle fixed into a

syringe of 2 or 5 ml capacity is inserted into the vein. When the needle enter the vein the plunger (pistan) of the syring is with drawn slightly, If the blood appear to come out, the tourniquet is released after drawing the desired amount of the blood a small pad of cotton wool soaked with sprit is placed on the arm where the needle was enserted and then the needle is with drawn. The cotton pad is held firmly for a few minutes until the bleeding stops. The needle is removed from the syringe and the blood is transferred to the vial by gently pushing the peston. Do not force the piston to expel the blood. This may cause haemolysis.

3.11.2. Jugular Vein :

Jugular vien runs over the trachea and carotid artery on both the sides of the neck in the neck in the Jugular groove.

The hair are clipped and the area is sterilized with spirit or 70% alcohal or any other antiseptic.

Let the antiseptic dry before the vien is punctured. A 18 gauge needle is suitable for goat, sheep and cow. The vein should be raised either by applying first by an assitant or tourmniquet ventral to the point of punture. Thevien should be felt with the help of finger by topping.

The needle is inserted in the vein by force in an angle avoiding through and through puncture of the vien.

The blood can be collected directly in the collection tube in case of cow. Whereas a syring may be used while collecting blood from, sheep & goat

The pressure is released before removing the needle fromthe vien to avoid the formation of haematoma. If the blood from jugular vien is to be collected in the casted mammals then the neck should be given a turn to upper side.

This help in distending the vein for puncture.

3.11.3 Ear Vein :

When only small amount of blood say 1 or 2 ml, is required, it can be collected from the ear vein. This method can be used in Cow (Cattle). The area is clipped and well sterilized over the vien on the dorsal aspect of the ear. The vein is distended either by applying pressure with the help of the hand using thumb and index finger or by applying an intestinal forceps or the forceps meant for this purpose. The blood is collected in syringe with the help of a 20 or 22 guage needle.

3.11.4. : Cephalic Vein :

The blood collecting from the cephalic vein. The area is well disinfected with 70% alcohol or any other antiseptic. The alcohol must evaporate completely before the needle is inserted.

In the case of dog, the hair should be clipped on the interior surface of the fore limb over the vein.

The vein is raised by applying pressure with the help of hand done by an assistant or by applying tourniquet around the elbow. The skin over the vein should be tensed with the help of the hand and the thumb of the left hand should be put on the left side of the vein to emmoblige the vein properly. The vein should be felt before

enserting the needle with the help of finger A 20 or 22. gauge needle attached to a 2 ml or 5 ml dry syring is alright for dog.

The syreing should not be dried with etheras it causes haemolysis. The needle should be inserted from the right side of the vein and one must stop for a which after penetrating the sking and subcutaneous tissue. Insert the needle in the vein where usually a click like noise is heard when needle penetrates the vein collect the blood according to the requirement.

The pressure over the elbow should be release before taking out the needle so as to avoid the formation of haematoma. The vein at the puncture side is pressed in between the thumb and index finger twisting it slightly after taking out the needle. Apply some antiseptic. Put the blood in the collection tube after removing the needle from the syringe as some of the cells may be broken if the blood is forced through the needle.

Clean the syringe just after collecting the blood in all the cases.

3.11.5 Recurrent Tarsal Vein :

The technique is same for collection of blood from the recurrent tarsal vein on the hind limb.

In this case the pressure should be applied on the stifle joint and the needle should be inserted on the lateral side of the hock joint where the vein takes a round.

3.12 Storage of Blood Sample :

(i) It is desirable to start the examination of sample within an hour of collection, but of it is to be delayed, the sample may be stored in refrigerator for 24 hours without any change in

the cell count. When a refrigerated sample is examined. It should be taken out from the refrigerator at least half an hour before the start of test. This bring the sample to room temperature. The blood is then mixed vigorous shaking is avoided to prevent haemolysis.

(ii) The sample can also be stored for a few hours in airtight boxes where ice is packed around the sample.

3.13 Prevention of Coagulation and use of anticoagulants :

A number of anticoagulants can be used for haematological purposes. The blood should be mixed gently with anticoagulant either by rotating the tube in between the two plams or by gentle inversion. Uigorous shaking should be avoided as. It may result in breakage of cells and production of foam. Following are some of the anticoagulants which can be used for haematological examination.

3.13.1 Patassium and ammonium oxalate combination :

This can be prepared by dissolving 8 gm of Patassium oxalte and 12 gm of Ammonium oxlate in one litre of distilled water one ml of this solution is sufficient for 10 ml of blood.

The vials can be prepared before hand which are dried in over or incubator at 70°C. This anticoagulants may be used for haemato crit value, hemoglobin estimation.

Ammonium - Potassium oxalate tablets are also available and one tablets are also available and one table is sufficient for 5 ml of blood.

3.13.2 Lithium Oxalate :

This has been recommended at a rate of 1.5 mg/ml when blood urea nitrogen are to be determined.

3.13.3 E.D.T.A. (Disodium Salt of ethylene diaminetetra acetic acid : It may be used at the rate of 1.0 to 2.0 mg/ml of blood. This is considered as one of the best anticoagulants for blood studies. It is also available in the form of tablets (E.D.T.A.P. Tablets) where one tablets is sufficient for 5 ml of blood.

3.13.4. Sodium Citrate :

Citrates are good for formation of red cell hemolysate. The solution is prepared by adding 3.8 gm of sodium citrate to 100ml of distilled water. 0.1 ml of his solution is sufficient for 10 ml of blood. One part of a citrate solution containing. The composition of ACD mixture is cetric acid ($C_6H_8O_7$. $2H_2O$) = 0.48%

Sodium citrate ($C_6H_5O_7$ Na_3. $2H_2O$ = 1.32%

Dextrose (C_6 H_{12} O_6. H_2O) = 1.4%

Water (H_2O) = 100 ml

3.13.5. Sodium Fluoride :

It is used at the rote of 1 mg / ml of blood. It inhibits glycolysis. As such it is very useful for blood suger estimation.

3.14 Preparation of red cell haemolysate :

Red cell haemolysate was prepared from the method followed by Beutler (1984a). The obtained red cells from 1ml blood sample by the above mention procedure were suspended in 0.154 M NaCl.

To one valume generally (0.1 ml) of packed red cells one valume of 0.154 m NaCl was added.

To this (0.2 ml) red cell suspension 1.0 ml of β-mercaptoethanol - EDTA the stabeliging solution prepared by bringing 0.05 ml of b-mercaploethane and 10 ml of neutralized 10% EDTA to a volume of 1 litre with water was added.

The tube containing the haemolysate was frozen over night and throwed by placing the tube in a beaker containing water at room temperature when the haemolysate was completely thawed, it was uniformly shaken and tube was kept in ice water where it was maintained at 0°C. The haemolysate prepared in this way was referred to as 1:20 haemolysate.

3.15 Estimation of Haemoglobin :

The estimation of haemoglobin was performed following the procedure of Beutler (1984a). Ferricyanide - cyanide reagent (Drabkin Solution) prepared by dissoving 100 mg NaCN and 300 mg K3fe(CN)6 in 1 litre of water. For determination of haemoglobin 0.2 ml hemolysate was added to 10 ml ferrieyanide - eyanide reagent.

Ferricyanide - cyanide regent when added to diluted hemoglobin converts hemoglobin to cyano methemoglobin and the optical density was measured at 540 nm within 5 minutes.

The spectrophotometer was calibrated by using commercially available cyanomethemoglobin standard (60mg/100ml) such a standard diluted in ferricyanide cyanide reagent to provide a concentration from 0.009 - 0.045 mg / 100 ml.

When the optical density of the diluted standard solution was plotted against their hemoglobin concentration A straight line was obtained. The observance volume (O.D. 540) at which the calibration plot intersects the hemoglobin concentration of 10 mg / 100 ml was designated A_1 and the hemoglobin calibration factor (FH6) was obtained as $1/100\ A_1$.

$F_{Hb} = 1/100A_1$

The concentration of haemoglobin in grams/100 ml in any sample is calculated by the formula.

Haemoglobin (g/100 ml) = ...

Where OD_{540} = Optical density at 540 nm wavelength

F_{Hb} = hemoglobin calibration factor

V_{fe} = Volume eyanide - ferricyanide solution used

V_{Hb} = Volume of haemoglobin solution used.

3.16 Estimation of haemoglobin by Haemoglobino meter :

Used haemo meter and haemo pippet. In haemometer take 20 μ l. N/10 Hydrochloric acid (HCl) and added 20 μl fresh blood then added with Distrilled water as require and match both side column (Standard) present already and read the reading from haemo meter.

3.17 Estimation the amount of Glucose :

The amount of glucose is estimated by the method followed by Nelson, Somogy's (1969). Blood is deproteinized by a Zinc hydroxide

Barium sulphate procedure, which give a filtrate containing practically no reducing substances other then glucose.

The Zinc-Barium filtrate is heated with an Alkaline copper reagent and then treated with a special Arsenomolybdate colur reagent. The colour developed is compared with that obtained from a known amount of glucose.

1. Neutralization of Barium hydroxide and Zinc-sulphate solution - 10 ml of Zinc - Sulphate Solution was measured into a flask and diluted with 50 ml distilled water. 4 Drops of Phenolphthalein solution water. 4 Drops of Phenolphthalein solution was added and treated with Barium hydroxide solution was used to get faint pink colour.

2. **Deproteinization :** 1.0 ml of Blood was placed in a flask and 9.5 ml of Barium bydroxide solution and 9.5 ml of Barium hydroxide solution and 9.5 ml of Zine - Sulphate Solution was added and mixed by rotation. Shook Vugorously and filtered on a dry filter paper and the filtrate was collected in a dry flask.

3. **Determination of glucose (Standard) :** Aliquest of 1 ml of glucose solution were taken to get a corresponding range upto 5 - 0 ml, 1.0 ml of Alkaline copper solution was aded in each tube and were placed in hot water both for 20 min. Then 1 - 0 ml of Arseno molybdate reagent was added in each tube to 10 ml with distilled water optical density was measured at 540 nm.

4. **Unknown :** To 0.5 ml. of Barium Zinc filtrate, 1 ml of Alkoline copper reagent was added and placed up right in

boiling water bath for 20 minutes. The 1.0 ml of Arseno molybdate reagent was added after colling and raised the valume to 10 ml with distilled H_2O. The photo metre was set to Zero density with the blank and measured the optical density of standard and unknown at 540 nm.

Suger acid $\rightarrow Cu^+ + OH^- \rightarrow CuOH \rightarrow Cu_2O$ Red ppt.

Cu_2O + Arsenomolybdate reagent $\rightarrow$ Blue colur Complex

Preparation of Reagents :

1. Arseno molybdate reagent - 11.0 g Ammonium molybdate was dissolved in 200 ml distilled H_2O. added 9.2 ml of conc. H_2SO_4.

2. 1.2. gm of Disodium ortho Arsenate was dissolued in 11 ml distilled H_2O.

3. Solution 1 & 2 was added and placed the mixture in an incuboter at 34°C for 2 days.

2. Alkaline Copper reagent :

Solution A - 12.5 g of anhydrous sodium carbonate + 12.5 g of Rochelle salt + 10 g of Sodium by carbonate + 1-- g of anhydrous sodium sulphate was dissolved in Distilled water & was diluted to 500 ml.

Solution B - 15 g of $CuSO_4 . 5H_2O$ was dissolved in 100 ml D. H_2O & added 0.5 ml of conc H_2SO_4.

Reagent - 4 ml of solution B was diluted to 100 ml with solution A.

3. Glucose Solution - (Stock Solution) - 10 mg glucose was dissolved in 100 ml of D. H_2O (100 µg/ml).

Working Solution - 10 ml of stock solution was raised upto 100 ml with D.H_2O (10 mg/ml)

4. **Barium Hydroxide Solution** - 22.5 gm of Ba $(OH)_2$. 8 H_2O was dissolved in 250 ml of D. H_2O.

5. **Zinc Sulphate Solution** - 12.5 gm $ZnSO_4$. 7 H_2O was dissolved in 250 ml of D. H_2O.

6. **Phenolpthlein reagent** - 500 mg phenolphtlein was dissolved and diluted in 50 ml ethyl alcohol.

3.18 Estimation of amount of glucose (By Kit) : Glucose is oxidized by glucose oxidase to gluconic acid and hydrogen per oxide. In a subsequent per oxidase catalyzed reaction the oxygen librated is accepted by the chromogen system to give a red coloured quinoneimine compound. The red colour so developed is measured at 505 nm and is directly proportional to glucose concentration.

3.19. Serum Glucose : 1 vial + 50 ml liquid glucose mix well and keep it 30 minutes in room temperature it becomes working solution for glucose test.

Take 1000 µl (1.0 ml) working solution in a cuvette and added 10 µl serum then incubate at 37°C for 15 minutes. Then sip in chemistry autoanalizer RA-50 and read the reading.

3.20 Determination of Protein : Protein was determined by the method of Lowry et. al (1951).

Falin's reagent consist of phaspho molybodic acid and phospho tungstic acid complex which is fored due to refluxing sodium tungstate, sodium molybdate and phosphoric acid.

In the first step, the protein is allowed to form a - copper protein complex with copper sulphate and in the second step copper protein complex formed reduced the phaspho molybdic phesphotungstic acid complex and blue colur is formed.

Preparation of Reagents :

Sodium hydroxidde (0.1 N) was prepared by the dissolving sodim jhydroxide (1 g) in glass - distilled water and the valume of made upto 250 ml.

Alkaline Copper Sulphate Solution :

(a) **Sodicum carbonate solution** (2% W/V) sodium carbonate (2g) was dissolved in sodium hydroxide (0.1 N) and the volume was made upto 100 ml.

(b) **Copper Sulphate Solution** (1% w/v) copper sulphate (1 g) was dissolved in glass distilled water and the volume was raised to 100 ml.

(c) **Sodium tart rate solution** (2% W/V) Sodium Tartrate (2.0 g) was dissolved in glass - distilled water and the volume was made upto 100 ml.

Alkaline copper sulphate solution was prepared by mixing 100 ml of solution (a) with 1.0 ml each of (b) and (c). This solution was prepared fresh daily just before use.

Trichloroacetic acid (10% W/V) was prepared by dissolving 10 g of the substances in glass - distelled water and raising to volume to 100 ml..

Folin's reagent - A mixture of sodium tungstate (50g) sodium molybdate (12.5 g) water (350 ml) phasphoric acid (50 ml 85% V/V) and conc. hydrochloric acid (50 ml) was refulxed in a one letre flask for 18 hours. Then lithium sulphate (75 g), water (25 ml) and few drops of bromine water were added to the flask.

The mixture was boiled to remove bromine vapours cooled the room temperature and diluted to 500 ml with glass - distilled water.

The reagent was titrated with sodium hydroxide (1 N) to Phenolphthalein end point. on the basis of titration, the reagent was diluted with water to make it equatorial with sodium hydroxide. The reagent was the stored in a refrigerator.

Standard Protein Soltion - (100 μg/ml) crystalline bovine serum albumin (10 mg) was dissolved in sodium hydroxide (0.1 N) and the volume was raised upto 100 ml.

Experimental Procedure :

Suitable aliquots (0.2 - 1.0 ml) were pipetted into a series of tubes and the volume was raised to 1 ml with sodium hydroxide (0.1 N). To each tube alkaline copper sulphate solution (5.0 ml) was added mixed well and allowed to stand at room temperature for 10 minutes. Folins reagent (0.5 ml) was then added, the contents of the tubes were mixed well immediately and after allowing to stand for 30 minutes the optical density was measured at 660 nm. A reagent blank and standard solution of protein (20 - 60 μg) were also run simultaneously for the calibration of curve.

3.21 Determination of Protein (By Kit) :

(Modified Biuret & Dumas method) Supplied by Span Dingnostics Ltd. Protein in serum react with copper of Beuret Reagent in alkaline medium to form a blue purple complex with absorption maximum at 550 nm.

Regents (Supplied in the kit)

Reagent 1 - Beuret Reagent (Modified)

Reagent 2 - Protein Standard.

Procedure -

Pipette into Cuvette	Blank (B)	Standard (S)	Test (T)
Serum	--	--	0.1 ml
Reagent 2 - Protein Standard	--	0.1 ml	--
Reagent 1 - Beuret reagent	5.0 ml	5.0 ml	5.0 ml

Mix well allow tubes to stand at room temperature for 5 minutes. Measure the optical density of standard (s) and test (T) an a calorie metre with a green fillter .

Calculation -

Serum Total Protein = T - B / S - B × Blank in

(g/ 100 ml)

Where T = Test

B = Blank

S = Standard

3.22 Determination of Albumin - (by Kit) :

(Modified Beuret & Dumas method 1971)

Albumin in serum binds with the dye ,Bromo Cresol green at pH 3.68 to form a gree coloured complex the absorbance of whcih is measured at 600 mm.

Reagents :

1. Buffered Dye Reagent
2. Protein Standard

Procedure :

Pipette into Cuvette	Blank (B)	Standard (S)	Test (T)
Reagent 1 - Buffered Dye reagent	4.5 ml	4.5 ml	4.5 ml
Serum	--	--	0.03 ml
Reagent 2 - Protein Standard	--	0.3 ml	--

Mix well, allow tubes to stand at room temperature 5 minuts. Measure optical density of standard and Test (T) with a red filter against Blank (B).

Calculation -

Serum albumin in g/100 ml= T - B / S - B × B.

Where = T = Test

B = Blank

S = Standard

3.23 Determination of Urea Nitrogen in Blood/Serum -

(This method was written by Rashid Ali)

Method for the determination of urea netrogen fall into categories. These are based on either the fearon reaction which is direct interaction of urea with diacetyle monoxime on those dependent upon hydrolysis by urease to produce ammonia which is subsequently measured. Numerous modification fo fearon's procedure have been introduced. Coulombe and favreau proposed use of thiosemicarbazide to stebilize and intensify the final colour produced by the diacetyle monoxime urea complex marsn et. al introduced a combination of ferric chloride and thiosemicarbazide for greater sensitivity.

Deprotinization of sample was eliminated by humninghake and crisolia.

The procedure described here is based essentially on that of crocker (1967) modified according to our laboratory condition.

Principle -

Urea reacts with diacetyl monoxime to form a pink product. The intensity of the complex is directly proportional to urea concentration. The complex is measured colorimetrically at 520 nm.

Diacetyl monoxime + urea → Pink

Chromongen + Hydrozylamine

Reagents :

1. Sulfuric acid - 10% (V/V)

2. Benzoic acid - (0.1%) 10 g. Dissolved in 1 litre of D. water.

3. Stock thiosemicarbazide - Dissolved 1.25 g thiosemi carbazide in D. water and dilute to 250 ml.

4. Stock Diacetyle monoxime - Dissolued 6.2 g diacetylmonoxime in D. water and dillate to 250 ml.

5. Stock Ferric chloride - Phasphoric acid dissolved 3.4 gm $FeCl_3$. $6H_2O$ in 67 ml orthophasphoric acid 85% dilute to 100 ml with water.

6. Stock urea nitrogen standard - (1.0 mg/ml) Dissolved 0.2143 g pure urea in 0.1 percent benzoic acid (as presevative) make up the volume to 100 ml.

7. Working acid FeCl3 - Dilute 1.3 ml stock ferric chloride to 1 litre with 10% Sulpuric acid.

8. Working diacetyle monoxime - mix 67 ml stock Deacetyl monoxime with 67 ml stock thiosemicarbazide and dilute to 500 ml with water.

9. Working urea Nitrogen standard - 1.0 mg / 100 ml. Dilute 1.0 ml of stock standard to 100 ml with 0.1 Percent benzoic acid.

Procedure - Take aliquotes 0.5 ml of working urea standard upto a maximum of 2.0 ml. raised the volume in each tube upto 2.0 ml with 1% Benzoic acid. Added 2.0 ml working Diacetylmonoxime Solution in each tube and mixed then added 3.0 ml of working FeCl3 mixed the solution and placed the tubes in a boiling water bath for 10 minutes. Remove and cooled the test tube under tap water & read the obsorbance at 520 nm.

3.24 Determination of Urea Nitrogen In Blood / Serum (By Kit) :

Urea condenses with O - Phthalaldehyde and Naphtyl ethylene diamine to form an orange coloured complex.

The rate of formation of this complex is directly proportional to urea concentration and is monitored on an initial roate (fixed time) mode at 505 nm.

Regents :

Reagent 1. - O phthal dehyde

Reagent 2 - NED

Reagent 3 - Urea Standard.

Procedure :

Pipette into Cuvette	Blank (B)	Standard (S)	Test (T)
Distilled Water	1000 µl	--	--
Reagent 1	--	1000 µl	1000 µl
Standard	--	50 µg	--
Serum	--	--	50 µl
Mixed Properly			
Reagent 2	--	500 µl	500 µl

1. Blank analyzer with Distilled Water

2. Aspirate Standard.

3. When standard result are printed prepare test and aspirate.

Calculation :Urea concentration mg/dl = T2 - T1 / S2 - S1 × 50

Where T = Test

S = Standard

3.25 Determination of creatinine :

The method discribed here is a modified version of Brod et. al (1948) Creatinie is treated with picric acid in alkaline medium, area colur develops which is measured colrimetically.

The reaction is not specific but at least over 85 percent colour is due to creatinine.

Reagents :

1. Sodium Tungstate (10%) - 10 g of $Na_2 WO_4.2H_2O$ per 100 ml water.
2. Sulphuric acid (2/3 N) - Dilute with gradual shaking 19 ml of concentrated sulphuric acid to 1 liter with D. water.
3. Sodium hydroxide - (10%) - 10 g NaOH per 100 ml. D. water.
4. Saturated picric acid solution - use Anal. R. Grade.
5. Stock creatinie standard - Dissolved 100 mg of pure dry creatinine in 100 ml 0.1 N-HCl (for 0.1 N. HCl) dilute 0.9 ml cone HCl to 100 ml with D. Water) the solution keeps well.
6. Working creatinine solution - Dilute 1.0 ml Stock solution to 100 ml with D. Water.
7. Alkaline picrate solution - prepare just before use a mixture of 10 ml Saturated picric acid and 2 ml sodium hydroxide.

Procedure :

Test - In a centrifuge tube measure 1.0 ml serum 4 ml D. water and add 0.5 ml sodium tungstate and 0.5 ml Sulfuric acid mix by in

version and centrifuge after some time. Take 3 ml supernatant in another test tube.

Standard :

3.0 ml working standard Blank - 3.0 ml water.

Blank - 3.0 ml D. water

Added - 1.5 ml alkaline picrate solution to each tube. Mix well and allow to stand for 10 minutes. Measure the obsorbance using green filter (520 nm) against the blank.

Calculation -

Serum Cretinie (mg/100 ml) = T/S × 6

Determination of Creatinine (By Kit) -

Creatinine forms a yellow orange compound in alkaline solution with picric acid.

At the low pecric acid concentration used in this method a prcipation of protein does not take place. The concentration fo the dyestuff form over a certain reaction time is a measure of the creatinine concentration.

As a result of the rapid reaction between creatinine and pieric acid, later secondary section do not couse an interference. This method thus distinguishes itself by its high specificity.

Reagents :

1. Buffer Solution -- 313 m mol/l of NaOH

12.5 m mol/l of phosphate

2. Pecric acid - 8.73 m mol/l of pecric acid

3. Standard Solution - 1 mg / dl = 88.4 μ mol/l creatinine.

Procedure :

Take 250 μl reagent 1 in an cuvette and added 250 μl reagent 2 in the cuvette wait for 10 minutes then added 100 μl serum and immediate sip the solution in a autoanalizer and read the reading.

3.27 Estimation of Total Cholesterol : The principle is based on the method of Zlatkis et. al (1993) for quantitative determination of cholestrol which based on the reagent containing $FeCl_3$, glacial acetic acid and cone H_2SO_4. Cholesterol gives a purple colour with these reagents. The colour is due to the preliminary dehydration to form 3.5, cholestadien and 2,4, cholestadiene which poly marized to give a dimer or trimer. The cholesterol due to polymer reaction coverts sulphuric acid to sulphonic acid which is represented as final products in the reaction.

Reagents :

1. Stock FeCl3 Solution - 2.5 g. of FeCl3 dissolved and raised to 100 ml phosphoric acid in dark.

2. Working FeCl3 Solution - 20 ml of stock was diluted ad raised to 250 ml with conc. H2SO4 in dark and in chilled condition.

3. Standard Cholesterol solution - A Stock was prepared by dissolving 100 mg Cholesterol in isopropanal and raised to 100 ml with isopropanal to get a solution of conc. 1 mg/ ml.

4. Working cholesterol solution - 25 ml of stock was diluted to 100 ml with isopropanal to get the solution of concentration of 250 μg / ml.

Procedure :

Extraction of cholesterol from serum take 5.0 ml of serum in cetnifuguge tube added 5 ml of iso propanol and centrifuged. To the supernatent 5.0 ml of isopropanal was added again and centrifuged.

The final supernatent was collected in a volumetric flask.

This was the clear extracted solution of cholesterol.

Estimation of cholesterol - A series of test were taken for different oliqots of working cholesterol solution. The volume in each tube was raised to 2.0 ml by isopropanal. So this was added 2.0 ml of glacial acetic acid followed by 2.0 ml of $FeCl_3$ solution with constant stirring in dark condition.

From Serum Supernatant - To 0.2 ml of serum supernatant was taken in a test tube and to it was added 1.8 ml isopropanol, 2.0 ml of glacial acetic acid and 2 ml of $FeCl_3$ solution in dark condition and take optical density at 625 nm.

3.28 Estimation of Total Cholesterol (By - Kit) - Followed by method Allain C.C. et. al (1974) :

Procedures :

Pipette into Cuvette	Blank (B)	Standard (S)	Test (T)
Serum	--	--	10 µl
Cholesterol Standard	--	10 µl	---
Working Cholesterol Reagent	1000 µl	1000µl	1000 µl

mix well, incubate at 37° for 10 minutes or Room temp. for 30 minutes.

1. Blank the analyzer with the reagent - Blank.
2. Aspirate Standard followed by tests.

Sip the solution in chemistry autoanalyzer RA-50 and read the reading :

3.29 To assay the activity of the enzyme alkaline phosphates - (EC. = 3.1.3.1) :

Alkaline phasphatase acts on buffered β-glycero phosphate (substrate) and librates phosphate (Pi) at pH 8.6 which when treated with ammonium molybdate forms phosphomolybdic acid which is subsequently reduced by ANSA to produced a blue coloured complex of phaspho molybdenum blue, whose intensity is directly proportional to the amount of phosphate (Pi) liberated, which can not read calorimetrically.

Preparation of reagents :

1. Stock Pi solution - Dissolved 43.9 mg KH2PO4 in D.H_2O and added 5.0ml of 10 NH_2SO_4 and rainsed to 100 ml by D.H_2O.

2. Working Pi solution- 10 ml of stock raised to 100 ml (10hg/ml)

3. Substrate solution-1.25 g. of β. glycero phasphate was dissolved and volume raised to 100'ml with D.H_2O : (12.5 mg/ml) the Ph of the Solution was adjusted to 8.6 by adding dil Hcl or Dol NaOH .

4. Acid molybdate solution - 5.0h of ammonium molybdte was dissolved in 40ml of D.H_2O & 60 ml of 10 N H2So4 was added to it and volume raised to 200 ml. with D.H_2O.

5. 30g% TCA. solution Dissolved 30g. T.C.A. and raised to the volume 100ml. with D.H_2O.

6. ANSA reagent - 500mg 1, 2, 4 ANSA was dissolved in 195 ml 15g% sodium Sulphite Solution .

7. 0.1NHcl - 0.4ml cone. Hcl was diluted to 46.8 ml with distilled water.

8. 0.1N NaOH - 0.4g NaOH dissolved in D.H_2O made the volume 100ml with distilled water.

Procedure :

Took 4 centrifuge tubes number then E,C,E_2 & C_2. In E_2 and C_2 took 9.0 ml of Alkaline substrate. Place all the tubes in on incubation at 37°C for 5 minutes. To the tube E_1 and E_2 add 1 ml of serum and keep all the tube in on encubator at 37°C for 1 hour.

Removes the tubes from incubation and keep it incise both for fear minutes (2 minutes and add 2.0 ml of 30% TCA in each tube to the tube, c and C_2 add 1 ml of serum follwed by 2 ml TCA and centrifuge all the tubes. discard the residue. take aliquot of supernatant for phosphorus estimation.

A series of tubes of standard experimental and control tubes developed In standard take Pi solution (0.5 to 2.0ml) followed by added 1.0ml Aaid molybate in each tube and ANSA also add 0.4 ml in each Tube and ANSA also add 0.4 ml in each tube mention 8.6 ml by distilled water. and from serum supernatant take aliquoles (0.5 to 1.0 ml) and all reagent followed the standard. Take optical density at 620 mm.)

To assay the activity of enzyme Alkaline phosphates (E.c - 3.1, 3.1) (By ket) : At pH 10.30 alkaline phosphates catalyzes the hydrolysis of p Nitrophenyl phasphate to yellow colured P-Nitrophenol and phasphate. Change in absorbance measured at 405 nm is directly proportion to enzyme activity.

Regaents :

1. Amp Buffer.
2. PNPP Substrate

PRCEDURE :

Pipette entocuvette	Blank	Test
Distilled water	1.0 ml	-
worked alkaline regent	-	1.0 ml
Serum	-	20hl.

Mix well and aspirate blank followed by test. Sip the reagent in outo analiger and read the reading.

3.31 To Estimation of Enzyme Lactate Dehydrogenase (LDH) EC - 1.1.1.27 :

The method serum is enccrebated with substrate, pyruvie acid the presence of co-enzyme $NADH_2$. some of Pyravic acid is converted to lactic acid by the action of LOH Present in the serum. The

remaining amount of pyruvic acid in alkaline medium to form pyrruvate hy dragoon which is measured calorimetrically. There fore in this method the decrease in colour of test in comparison to control gives the enzymatic activity.

Reagents:

1. Phosphate buffer (pH 7.4)- Dissolved 11g. of anhydrous disodium hydrogen phosphate per litre in water. Check and Adjust the pH.
2. Stock sodium pyruvate (37.5mM.) Dissolved 415 mg of sodium pyruvate in 100 ml of phosphate buffer. Divide into 1 ml samples and keep in refrigerator
3. Working sodium pyruvate buffered substrate (0.75mM) dilute 1 ml stock solution to 50ml with phosphate buffer make fresh dilution daily.
4. Reduced nicotinamide adenine dinucleotide ($NADH_2$) - 10 MG $NADH_2$ per ml of phosphate buffer. Make fresh daily.
5. 2-4 dinitrophenyle hydrazine (2mM) - Dissolved 400 mg of dinitro phenyl hydrazine in 85 ml of concentrated HCl make up to 1 litre with water and store in dark bottle.
6. Sodium hydroxide (0.4N)-16g NaOH per litre in water.

PROCEDURE :

Test : In a test tube take 1 ml of working substrate and 0.1 ml serum and keep in a water bath at 37°C for 3-5 minutes. Then add 0.1 ml of $NADH_2$ solution mix and incubate for exactly 15 minutes.

Remove the tube from water bath and immediately add 1 ml of dinetrophenyle hydrzine solution and mix.

Control : Mix 1 ml of working substrate 0.2 ml of buffer solution and 1 ml of dinitrophenyl hydrazine solution.

Blank : 1.2 ml buffer solution and 1 ml dinitrophenyl hydrazine solution.

Allow all the tubes to stand at room temperature for 20 minutes. Add 10 ml 0.4N-NaOH to each and mixed well. After 10 minutes measure the absorbency using green filter 540nm.

Calculation :

Serum lactate dehydrogenase = C-T/C-B x 500

Micro mol/min./lit. = IU/L

where C = Control

T = Test

B = Blank

3.32 ESTIMATION OF ENZYME LDH (by Kit) (E.C. 1.1.1.27) :

Lactate dehydrozgenase catalyzes the oxidation of lactate to pyruvate accompanied by the simultaneous reduction of NAD to NADH.

LDH activity in serum is proportional to the increase in observance due to the reduction of NAD.

Lactate + NAD $\xrightarrow{LDH}$ pyruvate + NADH .

Reagents :

1. Reagents 1- LDH co-enzyme
2. Reagents 2 -LDH Co-Buffered substrate

Working Reagent : One tablet of 1LDH with 1:1 ml of 2LDH mix gently to dissolved the contents use after 5 minutes.

Pipette into Text tube	**Test**
Working Reagent	1.0ml.
Serum	0.05ml.

Mix and read first observance of the test exactly at one minutes in outoanalizer.

3.33 ESTIMATION OF ENZYME SERUM CLUTAMIC - OXALOACETIC TRANG AMINASE : (SGOT) (This Is Applied By Reitman And Frankel 1957)

In SGOT determination, a substrate containing a mixture of Alpha-ketogluteric acid and aspartic acid is encubated with the serum. As a result of enzymatic action of SGOT on the substrate, glutamic acid and oxaloacetic acid are formed. Oxalo acetic acid is further decarboxylated and pyruvic acid is formed. The pyruvic acid is treated with 2-4 dinitrophenyle hydrazine in alkaline medium which form a brown cloured hydrozone which is measured colorimatrically.

Reagents : Phasphate buffer (pH 7.4) :

1. Dessolved 11.3 g of dry anhydrous disodium hydrozen phosphate (Na2 HPO4) and 2.7 g dry anhydruous potasium dihydrogen phosphate (KH4PO4) per litre in Distilled water mix well and check the pH with a pH metre store at 4^{0}C.

2. GOT substrate (200mM Dl aspartic acid 2mM alfa-ketoglutrate) Dissolved 13.3g. of DL-aspartic acid in about 90ml of n. NaOH solution. Add 0.146 of alfa ketoglutaric acid and dissolved it by adding a little more NaOH. Adjust the pH to 7.4 and make to 500 ml with phosphate buffer. Store in 4^{0}C in refrigerator.

3. Stock pyruvate standard (20mM) dissolved 220 mg of sodium pyruvate per 100 ml of phosphate buffer. Divide in 1ml portions and store at 4^{0}C in refrigerator.

4. Working pyruvate solution- (4mM) dilute 1ml of stock standard solution to 5ml with 4ml of phosphate buffer and store at 4°C in refrigerator prepare freshly every week.

5. 2-4 dimetre phenyl hydrazine phenyl hydrazine DNPH (1mM) dissolved 19.8mg of dinetro phenyle hydrazine in 10ml concentrated Hcl and make to 100ml with distilled water. Keep in a brown bottle at room temperature.

6. Sodium hydro rude(0.4H)- 16g of sodium hydroxide per litre Distilled water.

Procedure :

Test -take 0.5 ml of substrate in a test tube worn in a water bath at 37°C for 3 minutes then add 0.1 ml of serum, mix gently and incubate for 60 minutes exactly. Remove the Tubes from the bath and immediately add 0.5 ml of DNPH solution and mixe well.

Control- mix 0.5 ml of substrate with 0.5 ml of DMPH so lution and then add 0.1 ml of serum.

Standard - Mix 0.1 ml working pyruvolte standard with 0.4 ml of substrate, 0.1 ml of water and 0.5 ml of DNPH solution.

Blank - 0.5 ml substrate, 0.1 ml water and 0.5 ml of DNPH.

Allow the DNPH to react in all tubes for 20 minutes at room temperature.

Then add 5.0 ml of NaOH mix well and stand for 10 minutes.

Mesure the obserbance using the green filter (540 nm).

Calculation :

Micro mole pyruvale (ml/L serum) = T - C / S - B × 67 micro mole.

3.34 Estimation of Enzyme SGOT (by Kit) :

L-Asparted + α - Ketoglutarate == oxaloacetate + α-glutamatic

oxaloacetate + NADH + H == L. malate + NAD

There is a decrease in absorption at 340 nm as NADH in converted to NAD. The rate of decrease in observance is measured and is proportional to (Aspartate amiotransferase) activity in the sample.

Reagents :

Reagent 1 (Enzymes) → MDH, LDH, NADH, α - Ketoglutarote.

Reagent 2 - Tris buffer, PH 7.80, a-Asparted

Regent - Reconstitution -Dissolved the contents of one bottle of regante 1 with one bothle of reagent 1 A mix by gentle swirling.

Procedure :

Pipette into Text tube	Experiment
Reconstituted Reagent	1.0 ml.
Sample (Serum)	100 µl

Mix and sip in analizer and read the reading.

3.35 Estimation of Enzymeglutamic - Pyruvic Transminase (SGPT) : This is applied by Reitman and Frankel 1957 :

This enzyme can be estimated by the method Reitman and Frankel (1957). In SGPT determination - SGPT Substrate a mixture of a-ketogluteric acid and alanine is used and glutamic acid and pymxivic acid is formed. The pyrevic acid is treated with 2 - 4, dimtrophenylydrazine in alkaline medium which form a brown coloured hydragone which is measured colosimaticelly.

Regents :

1. GTP Substrate (200 m M alanine 2 mm a-ketoglutarate) - Dissolved I g of alanine in 90 ml water and added about 2.5 ml of N - NaOH to adjuest the pH to 7.4 . Add 0.146 g. of a-ketoglutaric acid dissolved it by adding a little more NaOH.

Adjust the pH to 7.4 and make to 500 ml with phasphate buffer. Store at 4°C All reagents are same as enzyme SGOT.

Procedure :

Procedure is the same as for SGOT except use GPT Substrote and encubote for only 30 minutes.

Calculation :

Micro mol pyruvate (mt/l serum) = T - C / S - B × 133 micro mole.

3.36 Estimation of Enzyme SGPT (By Kit)

L - Alanine + L - Ketogluturate == L - Gluumate + Pyruvate.

Pyruvate + NADH + H LDH L - Lactoate + NAD

There is a decrease in absorption at 340 nm as NADH is converted to NAD. The rate of decrease in observance is measured and is proportion to SGPT activity in the sample.

Reagents - Reagents 1. LDH, NADH and L - Ketoglutarate

Reagent (2) - This buffer pH 7.50 L - Alanine.

Reagent Reconstitution :

Dessolved the one contents bottle of Reagent 1 with reagent 2 min by gentle swirioning.

Procedure :

Pipette into Text tube	**Experiment**
Reconstituted Reagent	1.0 ml.
Sample (Serum)	0.05 µl.

Mix well sip in chemistry analizer RA-50 and read the reading.

* * * * * * *

Results & Discussion

RESULT & DISCUSSIONS

The mammals has to be maintain in a perfect state of health. Any deviation in health proportionaly alters the production To assess whether the mammals in a perfect health state or not, the biochemical analysis of blood sample in frequently employed.

Accordingly the metabolic profile test appears a good diagnostic tool for the early diagnosis and prediction of the production diseases.

The test need normal values of blood parameters from the population within the area.

Normal values of biochemical constituents of blood and serum in mammals are of academic as well as clinical importance. Extensive research conducted in India revealed that these value vary from region to region (Pandiya et. al. 1977). It is therefore necessary to establish the normal levels of biochemical constituents of blood and serum in mammals of particular region.

- **Haemoglobin level in different mammals :**

All the mammals examined were apparently healthy, active and alert with out any systemic disturbance and were in agreement with the observation of Rosenberger (1979). Blood collected in vial with sodium fluoride as anticoagulants and haemoglobin level were measured by calorimetrically at 540 nm. The procedure was the same as described by (Beutler 1984). Result are obtained in haemoglobin of normal condition of the mammals presented in (Table -1).

HAEMOGLOBIN LEVELS IN NORMAL CONDITION OF THE MAMMALS

Mammalian (Species)	Hb **Hg (g/dl)**
Human (Homo Sapiense)	15.60 ± 1.10
Goat (Capra Hircus)	10.80 ± 0.62
Sheep (Ovis aries)	13.40 ±1.20
Cow (Bos Indicus)	11.20 ± 1.40
Dog (Canis Familiaris)	14.50 ± 1.50

Sexual variation ?

Table No. 01 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

Significant difference ($P < 0.05$) in the haemoglobin content of different mammals was observed . The haemoglobin content shows wide differentiation in mammals. In human haemoglobin content were mean value (15.60 ± 1.10) g/dl in goat (capra - His cus) mean were (10.80 ± 0.62) g/dl in the sheep (ovis ovis) mean were 13.40 ± 1.20 g/dl) in where as in cow (Bos indicus) mean were 11.20 ± 1.40 g/dl and in Dog (canis familiaris) mean were 14.50 ± 1.50 g/dl.

The haemoglobin content in Human (Homo sapience) were higher than other mammals. Howkins et al. (1954) observed the ranged of haemoglobin in the human (15.1 ± 1.2) g/dl.

The haemoglobin content obtain were lowest in the Goat (Capra Hircus). The data show that Humen (Hemo-sapiese) require more haemoglobin content then other mammals and lowest haemoglobin content obtain in goat it means goat require minimum haemoglobin for their normal function of the body.

If we arranging the haemoglobin content mean of mammals the data obtained will be Humen (Homo sapiense) 15.60 ± 1.10 g/dl, Dog (Canis familiaris 14.50 ± 1.50, Sheep (ovis ovis). 13.40 ± 1.20 g/dl. In Cow (Bos indicus) 11.20 ± 1.40 g/dl and in the Goat (Capra Hircus) 10.80 ± 0.62 g/dl.

The mammals arranging on the basis of haemoglobin content in increasing order will be Humen (Homo Sapiense) > Dog (Canis familiaris) > sheep (ovis ovis) > Cow (Bos indicus) > Goat (capra hircus). Because haemoglobin was a very important protein and it has very importent role in the transport of oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs.

HAEMOGLOBIN LEVELS IN COLD CLIMATIC CONDITION OF THE MAMMALS

Mammalian (Species)	Hg. (g/dl.)	
	Normal Values	Cold Climatic Condition Values
Humen (Homo Sapiense)	15.60 ± 1.10	15.50 ± 1.16
Goat (Capra Hircus)	10.80 ± 0.62	10.60 ± 0.98+
Sheep (Ovis aries)	13.40 ±1.20	13.20 ± 1.16+
Cow (Bos Indicus)	11.20 ± 140	11.00 ± 0.15+
Dog (Canis Familiaris)	14.50 ± 1.50	14.40 ± 1.80

Table No. 02 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ P < 0.05 (Significant difference)

++ P < 0.01 (Significant difference)

So it is related to the respiratory system. If the mammals contain much content of Haemoglobin in their body utilized more oxygen then lower haemoglobin content mammals and carbon dioxide also evolved more than lower content haemoglobin mammals.

The comparative study of haemoglobin of different mammals (Table-1) shows some interesting correlation among mammals. A distinct demarcation exist between herbivorous (goat, sheep cow) and omnivorous (Human and Dog).

Yet all mammals are homeo thermic and ureotelic. All these correlation suggest adaptive changes in haemoglobin level in response to changing physiological and environmental conditions.

➢ The haemoglobin content obtained in the cold climatic condition presented in the Table 2.

This data also show the wide significant difference in haemoglobin level of the mammals in case of goat, sheep, and cow but non significant difference in case of Humen and Dog.

In Goat, Sheep & Cow in cold climatic condition the level decreases, but in case of human and dog it remains same the mean of cold climatic data were - Humen (Homo sapience) 15.54 ± 1.16 g/dl. Goat (Capra Hircus) 10.60 ± 0.98 g/dl, Sheep (ovis arise) 13.20 ± 1.16 g/dl. Cow (Bos indicus) 11.00 ± 0.15 g/dl. and Dog (Canis familiaris) 14.40 ± 180 g/dl., the increasing order also will be.

Humen > Dog > Sheep > Cow > goat. This low level of haemoglobin a long standing low protein status and nutritional deficiency in the cold climatic condition then normal condition.

➢ The haemoglobin content obtained in hot climatic condition presented in the Table 3.

This data also shows the wide variation in case of goat, sheep and cow significant difference ($P < 0.05$) in hot climatic condtion. Then normal condition of the mammals. The haemoglobin level were increase in hot climatic condition, in case of goat, sheep & cow but in humen & dog it shows non significant difference. The mean hot climatic condition data were. Human (Homo sapinse) 15.00 ± 1.15 g/dl. Goat (Capra Hircus) 11.50 ± 0.82^{+}, Sheep (ovis ovis) 13.60 ± 1.40^{+} g/dl, Cow (Bos indicus 11.40 ± 1.60^{+} g/dl & Dog (Canis familiaris) 14.00 ± 1.70 g/dl the increasing order of haemoglobin content then normal will be Human > Dog > Sheep > Cow > Goat.

The haemoglobin level increase in goat, sheep and cow due to the water deprivation under thermal stress which ultimately resulted in haemo concentration.

➢ The haemoglobin content also observed in different climatic condition such as monsoon (from July to September) Winter (December & January) and summer (May & June) the data presented in the Table 4.

The haemoglobin level has wide variation in different climatic condition (in goat, sheep & cow significant difference ($P < 0.05$) but in Human and dog ($P < 0.01$) significance difference.

The mansoon its also known normal condition the haemoglobin level found significant difference ($P < 0.05$) in different mammals (goat, sheep and cow). But non significance change in humen and dog. But in the winter which is known as cold climatic condition the haemoglobin level found significant difference

HAEMOGLOBIN LEVELS IN HOT CLIMATIC CONDITION OF THE MAMMALS

Mammalian (Species)	Hg. (g/dl.)	
	Normal Values	Hot Climatic Condition Values
Human (Homo Sapiense)	15.60 ± 1.10	15.70 ± 1.65
Goat (Capra Hircus)	10.80 ± 0.62	10.30 ± 0.82+
Sheep (Ovis aries)	13.40 ±1.20	13.60 ± 1.40+
Cow (Bos Indicus)	11.20 ± 140	11.40 ± 1.60+
Dog (Canis Familiaris)	14.50 ± 1.50	14.60 ± 1.70

Table No. 03 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ P < 0.05 (Significant difference)

++ P < 0.01 (Significant difference)

HAEMOGLOBIN LEVEL IN DIFFERENT SEASION (DIFFERENT CLIMATIC CONDITION) OF THE MAMMALS

Mammalian (Species)	Different Season (g/dl.)		
	Mansoon[1]	Winter[2]	Summer[3]
Humen (Homo Sapiense)	15.60 ± 1.10	15.50 ± 1.16	15.70 ± 1.15++
Goat (Capra Hircus)	10.80 ± 0.62	10.60 ± 0.98+	11.50 ± 0.82+
Sheep (Ovis aries)	13.40 ±1.20	11.00 ± 1.16+	13.60 ± 1.40+
Cow (Bos Indicus)	11.20 ± 140	14.40 ± 0.15+	11.40 ± 1.60+
Dog (Canis Familiaris)	14.50 ± 1.50	14.20 ± 1.80	14.60 ± 1.70++

Table No. 04 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ P < 0.05 (Significant difference)

++ P < 0.01 (Significant difference)

1 –

2 –

3 –

($P < 0.05$) than normal values. In case of Goat, sheep and cow but non significance human & dog. Where as in the summer (Hot climatic condition. The haemoglobin level (Significance increase in goat, sheep and cow due to the water deprivation under thermal stress. Which altimatly resulted the haemo concentration and these mammals body weight also reduced about 0.2 Kg. (2% of body wt.) but in the case of humen and Dog their haemoglobin significant difference ($P < 0.01$).

All these correlation among the mammals suggest adaptive change in haemoglobin level in response to changing physiological and environmental condition.

➢ Haemoglobin level also observed in different age group of the mammals their data is presented in the table 5-8.

In the case of human (Hemo sapience) the haemoglobin level in child hood (age group 1 - 5 year) and Adult stage (21-30 year) non significantly difference but their mean of values were 15.73 ± 1.17 g/dl and 15.60 ± 1.10 g/dl but in comparatively old age the haemoglobin level significantly difference ($P < 0.05$) their mean of value were 13.56 ± 1.04 g/dl. In the case of goat (Capra Hircus) different age group haemoglobin also observed.

The age group 3 month to 1 years age group mean of haemoglobin value were 10.96 ± 0.24 g/dl and age group 2 - 5 years haemoglobin value mean were 10.80 ± 0.62 g/dl and old age group value is 9.18 ± 0.82 g/dl.

On comparative age group haemoglobin level the age group childage and Adult has non significant difference but in old age group have significant difference ($P < 0.05$).

HAEMOGLOBIN LEVEL IN CHILD AGE GROUP OF THE MAMMALS

Mammalian (Species)	Age group	Haemoglobin level (g/dl.)
Humen (Homo Sapiense)	1 - 5 Years	15.73 ± 1.17
Goat (Capra Hircus)	3 months - 1 years	10.96 ± 0.24
Sheep (Ovis aries)	3 months - 1 years	13.75 ± 1.02
Cow (Bos Indicus)	3 months - 2 years	11.35 ± 1.01
Dog (Canis Familiaris)	3 months - 1 years	14.62 ± 1.43

Table No. 05 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

HAEMOGLOBIN LEVEL IN ADULT AGE GROUP OF THE MAMMALS

Mammalian (Species)	Age group	Haemoglobin level Hg. (g/dl.)
Humen (Homo Sapiense)	21 - 30 Years	15.60 ± 1.10
Goat (Capra Hircus)	2 - 5 years	10.80 ± 0.62
Sheep (Ovis aries)	2 - 5 years	13.40 ± 1.20
Cow (Bos Indicus)	3 - 8 years	11.20 ± 1.40
Dog (Canis Familiaris)	2 - 5 years	14.50 ± 1.50

Table No. 06 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

HAEMOGLOBIN LEVEL IN OLD AGE GROUP OF THE MAMMALS

Mammalian (Species)	Age group	Haemoglobin level Hg. (g/dl.)
Humen (Homo Sapiense)	above 60 years	13.56 ± 1.04
Goat (Capra Hircus)	above 5 years	09.18 ± 0.82
Sheep (Ovis aries)	above 5 years	11.13 ± 1.07
Cow (Bos Indicus)	above 8 years	09.12 ± 1.20
Dog (Canis Familiaris)	above 5 years	12.14 ± 1.16

Table No. 07 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

HAEMOGLOBIN LEVEL IN DIFFERENT AGE GROUPS OF THE MAMMALS

Mammalian Species	Age groups	Haemoglobin Level (g/dl)
Humen (Homo Sapiense)	1 - 5 Years	15.73 ± 1.17
	21 - 30 Years	15.60 ± 1.10
	Above 60 Years	13.56 ± 1.04+
Goat (Capra Hircus)	3 months 1 years	10.96 ± 0.24
	2 to 5 years	10.80 ± 0.62
	above 5 years	09.18 ± 0.82+
Sheep (Ovis aries)	3 months to 1 years	13.57 ± 1.02
	2 - 5 years	13.40 ± 1.20
	above 5 years	11.13 ± 1.07+
Cow (Bos Indicus)	6 month to 2 years	11.35 ± 1.01
	5 - 8 years	11.20 ± 1.40
	above 8 years	9.12 ± 1.20+
Dog (Canis Familiaris)	3 months to 1 years	14.62 ±1.43
	2 to 5 years	14.50 ± 1.50
	above 5 years	12.14 ± 1.16+

Table No. 08 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ P < 0.05 (Significant difference)

++ P < 0.01 (Significant difference)

In the case of sheep, cow and dog. All mammals have age group childage and Adult values have non significant difference but in the case of old age their haemoglobin level decreases and significant difference ($P < 0.01$) due to the protein deficiency and lack of less amount of haemoglobin formation in old age of the mammals.

➢ The haemoglobin level also observed in 60 days of protein rich diet in the mammals. This is presented in table 9.

The haemoglobin level not significant different shows in any mammals then normal values.

Their mean of value were - (Homo - sapience) 15.50 ± 1.40 g/dl, Goat (Capra Hircus), 11.20 ± 0.08 g/dl, sheep (ovis ovis) 13.30 ± 1.60 g/dl, Cow (Bos indicus) 11.10 ± 1.60 g/dl, Dog 14.46 ± 1.83 g/dl.

Because protein rich diet not have very much effect in haemoglobin production of the mammals.

➢ The haemoglobin level also observe in grazing mammals. Which was presented in Table - 10.

The mean of their value were Goat (Capra hircus) 7.60 ± 1.02 g/dl., Sheep (ovis ovis) 9.78 ± 0.86 g/dl, Cow (Bos indicus) 8.18 ± 0.09 g/dl.

These values have wide variation then normal values the haemoglobin level much significant difference ($P < 0.05$) Compression to normal value the lower value of haemoglobin (Hb) due to the nutritional deficiency.

EFFECT OF PROTEIN RICH DIET IN HOEMOGLOBIN LEVEL OF DIFFERENT MAMMALS

Mammalian (Species)	Hg. (g/dl.)	
	Normal Values	Protein Rich Diet Values
Humen (Homo Sapiense)	15.60 ± 1.10	15.50 ± 1.40
Goat (Capra Hircus)	10.80 ± 0.62	11.12 ± 0.08
Sheep (Ovis aries)	13.40 ±1.20	13.20 ± 1.60
Cow (Bos Indicus)	11.20 ± 1.40	11.10 ± 1.60
Dog (Canis Familiaris)	14.50 ± 1.50	14.46 ± 1.83

Table No. 09 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

HOEMOGLOBIN LEVEL IN GRAZING MAMMALS

Mammalian (Species)	Hg. (g/dl.)	
	Normal Values	Value in grazing mammals
Goat (Càpra Hircus)	10.80 ± 0.62	7.60 ± 1.02
Sheep (Ovis aries)	13.40 ± 1.20	9.78 ± 0.86
Cow (Bos Indicus)	11.20 ± 1.40	8.18 ± 0.09

Table No. 10 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ P < 0.05 (Significant difference)

++ P < 0.01 (Significant difference)

MC Dowell et. al 1983, reported that grazing in tropical countries can rarely satisfy all minerals reqirements for live stock and Sarkar et. al (1992).

➢ The haemoglobin level were also observed in the different lactating mammals. Their values presented in Table 11.

Haemoglobin content was lower on the day of kidding, 3rd day and 90th day then 7th and 30th day of lactation.

Lower haemoglobin content on the day of Kidding, 3rd day and 90th day then 7th and 30th day of lactation (Table 8) because of added requirement of full term fetus in former and energy drain from body in peak lactation in the later situation.

Blood glucose level in different mammals :

The blood glucose level were observe in different mammal. The blood was collected in vial with sodium flouide (1 mg/ml) as anticoaylent It inhibits glycolysis.

The method follow by Nelson, Somogyi (1969) and by Kit Trinder P (1969), Tietz N.W. (1976).

➢ Blood glucose content obtain in normal condition of the mammals present in Table 12.

Significant difference ($P < 0.05$) in blood glucose level observed in different mammals.

In human (Hemo sapiense) blood glucose content mean were 89.40 ± 5.60 mg/dl. In goat (Capra Hircus) mean value were 63.00 ± 4.70 mg/dl. Sheep (ovis ovis) mean value were 46.20 ± 5.80 mg/dl

HAEMOGLOBIN LEVEL DURING LACTATING IN DIFFERENT MAMMALS

Lactating Days	Goat	Sheep	Cow
Day of Kidding 1	9.20 ± 1.60	13.20 ± 1.20	11.20 ± 1.40
3rd day of lactating	9.40 ± 1.80	13.40 ± 0.82	11.40 ± 1.20
7th day of lactating	9.70 ± 1.90	13.70 ± 0.85	11.70 ± 1.30
30th day of lactating	9.30 ± 1.10	13.30 ± 0.91	11.30 ± 1.10
90th day of lactating	8.90 ± 1.50	12.80 ± 0.22	10.80 ± 1.00

Table No. 11 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

BLOOD GLUCOSE LEVEL IN NORMAL CONDITION OF THE MAMMALS

Mammalian (Species)	**Blood Glucose (Mg/dl)**
Humen (Homo Sapiense)	89.40 ± 5.60
Goat (Capra Hircus)	63.00 ± 4.70
Sheep (Ovis aries)	46.20 ± 5.80
Cow (Bos Indicus)	55.00 ± 4.50
Dog (Canis Familiaris)	76.00 ± 6.80

Table No. 12 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

where as cow (Bos indicus) mean value were 55.00 ± 4.50 mg/dl and Dog (Canis familiaris) mean value wer 76.00 ± 6.80 mg/dl.

The blood glucose content were higher then other mammals.

Hlad et. al (1949) observe the range of blood glucose in the humen mean value (88.3 ± 6.7) mg/dl.

The blood gluose content obtained were lowest in sheep (ovis ovis). the data (Table 12) shows that humen require more glucose content then other mammals and lowest blood glucose obtained in sheep means sheep require minimum glucose content for their normal function of the body.

Because Blood glucose is a energy providing substances. One molecule of glucose after complete oxidation to produce 38 ATP. The energy most useful for normal function of the body. Regarding this point Trivelli et. al (1971) Koinig et. al (1977) and Bunn (1981) have reported that glucose reacts with haemoglobin non enzymatically with the NH_2 terminal residue of β-Chain (β - NaI valine) to form glycosylated haemoglobin.

If we arranging the glucose content mean of the mammals (Table 09) increasing order the data will be humen (Hemo sapience) 89.40 ± 5.60 mg/dl. Dog (caris familiaris) 76.00 ± 6.80 mg/dl. Goat (Capra - Hircus) 63.00 ± 4.70 mg/dl. Cow (Bos indicus) 55.00 ± 4.50 mg/dl. and sheep (ovis ovis) 46.20 ± 5.80.

The mammals arranging on the basis of glucose content in increasing order Humen > Dog > Goat > Cow > Sheep.

➢ Blood glucose content obtained in the cold climatic condition present in the Table - 13.

BLOOD GLUCOSE LEVEL IN COLD CLIMATIC CONDITION OF THE MAMMALS

Mammalian (Species)	Blood Glucose (Mg/dl)	
	Normal Values	Cold Climatic Values
Human (Homo Sapiense)	89.40 ± 5.60	88.40 ± 5.10
Goat (Capra Hircus)	63.00 ± 4.70	63.20 ± 3.60
Sheep (Ovis aries)	46.20 ± 5.80	45.00 ± 5.70
Cow (Bos Indicus)	55.00 ± 4.50	54.20 ± 4.20
Dog (Canis Familiaris)	76.00 ± 6.80	77.00 ± 6.00

Table No. 13 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ P < 0.05 (Significant difference)

++ P < 0.01 (Significant difference)

The data obtained in cold climatic condition have non significant difference then normal condition of the mammals.

The mean of cold climatic data were Humen (Homo Sapiense) 88.40 ± 5.10 mg/dl, Goat (Copra Hircus) 63.20 ± 3.60 mg/dl., Sheep (ovis ovis) 45.00 ± 5.70 mg/dl., Cow (Bos indicus) 54.20 ± 4.20 mg/dl. and dog (canis familiaris) 77.00 ± 6.00. Their in creasing order of blood glucose content was same.

Blood glucose level obtain in Hot climatic condition presented in Table 14.

The data obtained in hot climatic condition have wide variation then normal condition.

Significant difference ($P < 0.05$) in each mammals.

The mean of hat climatic date were Humen (Homo sapience) 99.70 ± 5.30 mg/dl, Goat (Capra hircus) 73.40 ± 5.60 mg/dl., Sheep (ovis ovis) 56.40 ± 6.60 mg/dl. Cow (Bos endicas) 65.20 ± 4.80 mg/dl and Dog (Canis familiaris) 86.10 ± 6.90 mg/dl.

In arranging the increasing order of mean value of blood glucose content is some as normal condition such as Humen > Dog > goat > cow > sheep.

The blood glucose level also obtained in different climatic conduction of the mammals it present in Table 15.

In monsoon known as normal condition their in non significant difference then cold climatic condition in blood glucose level of the mammals.

BLOOD GLUCOSE LEVEL OF IN HOT CLIMATIC CONDITION OF THE MAMMALS

Mammalian (Species)	Blood Glucose (Mg/dl)	
	Normal Values	Hot Climatic Values
Humen (Homo Sapiense)	89.40 ± 5.60	99.70 ± 5.30
Goat (Capra Hircus)	63.00 ± 4.70	73.40 ± 5.60
Sheep (Ovis aries)	46.20 ± 5.80	56.40 ± 6.60
Cow (Bos Indicus)	55.00 ± 4.50	65.20 ± 4.80
Dog (Canis Familiaris)	76.00 ± 6.80	86.10 ± 6.90

Table No. 14 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

BLOOD GLUCOSE LEVEL IN DIFFERENT SEASION (DEFFERENT CLIMATIC CONDITION) OF THE MAMMALS

Mammalian (Species)	Blood Glucose level in (Mg/dl)		
	Mansoon[1]	Winter[2]	Summer[3]
Humen (Homo Sapiense)	89.40 ± 5.60	88.40 ± 5.10	99.70 ± 5.30
Goat (Capra Hircus)	63.00 ± 4.70	62.20 ± 3.60	73.40 ± 5.60
Sheep (Ovis aries)	46.20 ± 5.80	45.00 ± 5.70	56.40 ± 6.60
Cow (Bos Indicus)	55.00 ± 4.50	54.20 4.20 ±	65.20 ± 4.80
Dog (Canis Familiaris)	76.00 ± 6.80	77.00 ± 6.00	86.10 ± 6.90

Table No. 15 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ P < 0.05 (Significant difference)

++ P < 0.01 (Significant difference)

But significance difference ($P < 0.05$) observed in hot climatic condition (summer) then monsoon in glucose level of the mammals.

The blood glucose level also observed in different age group of the mammals, human (Homo sapience), goat (capra hircus), Sheep (ovis ovis) cow (bos indicus) and Dog (canis familiarise) which is presented in Table 16-19.

Blood glucose level have non significant difference in age group childhood (1 - 5 years to Adult age (21-30 year). But wide variation were observed in blood glucose level in old age (above 60 years) significant difference ($P < 0.01$) decrease the blood glucose level then childhood and adult.

Narval et. al (1945) and Hald et al (1956) obtain the blood glucose level mean value in childhood and Adult 7.4 ± 15 and 88.3 ± 6.7 respectively.

Same value also found in case of Child and Adult goat (Capra Hircus) sheep (ovis ovis) Cow Bos inducs) and Dog (Canis familiaris and same significant difference ($P < 0.01$) also observed in old age of goat, sheep cow and dog.

- Blood glucose level also observed in after protein rich diet in 60 days of the mammals. The data obtained were presented in Table 20. The protein feeding segnificantly ($P < 0.05$) increased the higher blood glucose level at 60 days feeding in each mammals.

In general, this is in agreement with finding of others (Bassett 1972, 1974, Borrebeck et. al 1990, Amaral - Phillips et. al 1993.

The compression between mammals indicates a direct relation ship between the level of food intake and the plasma concentration

BLOOD GLUCOSE LEVEL IN CHILD AGE GROUP OF THE MAMMALS

Mammalian (Species)	Age group	Haemoglobin level (g/dl.)
Humen (Homo Sapiense)	1 - 5 Years	86.32 ± 5.43
Goat (Capra Hircus)	3 months - 1 years	61.14 ± 4.26
Sheep (Ovis aries)	3 months - 1 years	45.32 ± 5.36
Cow (Bos Indicus)	3 months - 2 years	53.68 ± 4.23
Dog (Canis Familiaris)	3 months - 1 years	72.18 ± 6.23

Table No. 16 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

BLOOD GLUCOSE LEVEL IN ADULT AGE GROUP OF THE MAMMALS

Mammalian (Species)	Age group	Blood glucose level (mg/dl.)
Humen (Homo Sapiense)	21 - 30 Years	89.40 ± 5.60
Goat (Capra Hircus)	2 - 5 years	63.00 ± 4.70
Sheep (Ovis aries)	2 - 5 years	46.20 ± 5.80
Cow (Bos Indicus)	3 - 8 years	55.00 ± 4.50
Dog (Canis Familiaris)	2 - 5 years	76.00 ± 6.80

Table No. 17 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

BLOOD GLUCOSE LEVEL IN OLD AGE GROUP OF THE MAMMALS

Mammalian (Species)	Age group	Blood Glucose (mg/dl.)
Humen (Homo Sapiense)	above 60 years	87.35 ± 5.21
Goat (Capra Hircus)	above 5 years	61.74 ± 4.32
Sheep (Ovis aries)	above 5 years	45.42 ± 5.316
Cow (Bos Indicus)	above 8 years	53.72 ± 4.35
Dog (Canis Familiaris)	above 5 years	74.01 ± 6.24

Table No. 18 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

BLOOD GLUCOSE LEVEL IN DIFFERENT AGE GROUPS OF THE MAMMALS

Mammalian Species	Age groups	Blood Glucose Level (mg/dl)
Humen (Homo Sapiense)	1 - 5 Years	86.32 ± 5.43
	21 - 30 Years	89.40 ± 5.60
	Above 60 Years	87.35 ± 5.21
Goat (Capra Hircus)	3 months 1 years	61.14 ± 4.26
	2 to 5 years	63.00 ± 4.70
	above 5 years	61.74 ± 4.32
Sheep (Ovis aries)	3 months to 1 years	45.32 ± 5.36
	2 - 5 years	46.20 ± 5.80
	above 5 years	45.42 ± 5.16
Cow (Bos Indicus)	6 month to 2 years	53.68 ± 4.23
	5 - 8 years	55.00 ± 4.50
	above 8 years	53.72 ± 4.35
Dog (Canis Familiaris)	3 months to 1 years	72.18 ± 6.23
	2 to 5 years	76.00 ± 6.80
	above 5 years	74.01 ± 6.24

Table No. 19 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

BLOOD GLUCOSE LEVELS OF IN PROTEIN RICH DIET OF THE MAMMALS

Mammalian (Species)	Blood Glueose (Mg/dl)	
	Normal Values	After protein rich diet (60 days)
Humen (Homo Sapiense)	89.40 ± 5.60	105.20 ± 4.80
Goat (Capra Hircus)	63.00 ± 4.70	79.00 ± 3.90
Sheep (Ovis aries)	46.20 ± 5.80	62.10 ± 4.80
Cow (Bos Indicus)	55.00 ± 4.50	71.00 ± 3.50
Dog (Canis Familiaris)	76.00 ± 6.80	92.00 ± 5.90

Table No. 20 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

of glucose. In under nutrition both rate of glucose synthesis turnover seen to decrease glucose synthesis probably decreases because of availability of propionate (Katz and Berg man 1969, Berg man 1973 where as glucose ten over is directly and proportionally affected by the reduction of glycemia (Bergman 1983) four to seven hours after the meal the glyconeogenesis rate is stillhigh compared with that observed in the post prendial period Katz and Bergman (169). Promoting the hyperglycemia observed.

➢ Blood glucose level observed in fat rich diet after 7 days of the mamals presented in the Table 21. The mammals Humen (Homo sapience), Goat (capra hircus), Sheep (ovis ovis), Cow (Bos inducs) and Dog (Canis familiaris). Were kept in individual pens and their ratien consisted of concentrated freed mixture according to their calculated reqiremnet.

Blood glucose level was physiologically of fat feeding means value in humen 81.60 ± 4.40, Goat 60.30 ± 5.70, Sheep 37.40 ± 5.60, Cow 46.10 ± 4.70 and in Dog 167.00 ± 5.90 then showed a trasient 9% decreases ($P < 0.05$) significant difference in each mammals.

➢ Blood glucose level also observed in grazing mammals which is presented in Table 22.

The mammals were supposed to be suffering from nutritional anemia.

The blood glucose level of grazing mammals were observed (Table 22). Segnificent difference ($P < 0.05$) decreases then normal values of the mammals (goat, sheep and cow) in goat 58.40 ±, sheep 41.60 ± 5.40 and cow 51.20 ± 4.80.

BLOOD GLUCOSE LEVEL OF IN FAT RICH DIET OF THE MAMMALS

Mammalian (Species)	Blood Glueose (Mg/dl)	
	Normal Value	After Fat rich diet value
Humen (Homo Sapiense)	89.40 ± 5.60	81.60 ± 4.40
Goat (Capra Hircus)	63.00 ± 4.70	60.30 ± 5.70
Sheep (Ovis aries)	46.20 ± 5.80	37.40 ± 5.60
Cow (Bos Indicus)	55.00 ± 4.50	46.10 ± 4.70
Dog (Canis Familiaris)	76.00 ± 6.80	67.00 ± 5.90

Table No. 21 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

BLOOD GLUCOSE LEVEL OF IN GRAZING MAMMALS

Mammalian (Species)	Blood Glueose (Mg/dl)	
	Normal Values	Value in grazing mammals
Goat (Capra Hircus)	63.00 ± 4.70	58.40 ± 4.60
Sheep (Ovis aries)	46.20 ± 5.80	41.60 ± 5.40
Cow (Bos Indicus)	55.00 ± 4.50	51.20 ± 4.80

Table No. 22 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

Higher prevalence of anemia recorded in grazing mammals suggest the possibility an acclimatized mamals of temperate type sweat profusely and loss of solvia and mucus from the mouth resulting in lose of significant quantities of minerals in tropical areas (Payne 1966).

The report on the present study indicated that massive cross breeding programme to boost up milk production in India could have failed to yield desired results and have had a greater impact over the other production aspects including indirect reproduction problem resulting to serious economic loss.

➢ Blood glucose level also observed in starvation period of the mammals (goat, sheep and cow) the data obtained were presented in table 23.

The blood glucose level non-significant in the mammals (goat (Capra Hircus), Sheep (ovis ovis), Cow (Bos indcus). But significant difference ($P < 0.05$) decreases in the case of human (Homo - Sapience and Dog (canis familiarise) the blood glucose level of mammals (goat sheep, and) were the same even after 24 hours of fasting.

In mono gastric mammals the glucose supplied the initial energy during starvation followed by a concomitant fall of blood glucose level. Through the valatile fatty acids (VFAS). Supply the initial energy, yet the significant role of blood glucose in ruminants during starvation cannot be ignored.

It is possible that unlike in monogastric mammals the on availability of substrate for microbial digestion did not take place in the ruminant during the short period of starvation and hance the

BLOOD GLUCOSE LEVEL OF IN STARVATION PERIOD OF THE MAMMALS

Mammalian (Species)	Blood Glueose (Mg/dl)	
	Normal Value	Starvation after 24 hrs. value
Humen (Homo Sapiense)	89.40 ± 5.60	80.60 ± 5.40
Goat (Capra Hircus)	63.00 ± 4.70	57.00 ± 4.80
Sheep (Ovis aries)	46.20 ± 5.80	40.10 ± 5.20
Cow (Bos Indicus)	55.00 ± 4.50	49.30 ± 4.70
Dog (Canis Familiaris)	76.00 ± 6.80	67.40 ± 6.60

Table No. 23 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

BLOOD GLUCOSE LEVEL IN LACTATING PERIOD OF THE MAMMALS

Lactating Days	Goat	Sheep	Cow
Day of Kidding	63.00 ± 4.70	46.20 ± 5.80	55.00 ± 4.50^{+}
3rd day of lactating	65.10 ± 4.60^{+}	48.30 ± 5.70^{+}	57.00 ± 4.60^{+}
7th day of lactating	63.20 ± 4.80	46.40 ± 5.60	55.20 ± 4.80^{+}
30th day of lactating	57.40 ± 4.60	41.60 ± 5.40	50.40 ± 4.80^{+}
90th day of lactating	73.10 ± 4.90	56.40 ± 5.60	65.80 ± 4.20^{+}

Table No. 24 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

withdrowal of food for 24 hours was not sufficient enough to induced hypoglycemia in these mammals (goat, sheep and cow). Blood glucose level also observed by author during course of research work in lactating mammals.

The data obtained were presented in the table 24.

Blood glucose content was however higher ($P < 0.01$) only on 90th day of kidding compared to other period in all mammals goat, sheep and cow.

Lower blood glucose content on the day of kidding and upto 30 days post kidding because of added requirement of full term fetus in former and energy drown from body in peak lactation in the later situation.

Serum protein level in different mammals :

- Serum protein level were observed in different mammals.

Serum protein level were measured by colorimetically at 660 nm.

The procedure was the same as describe by Folins Lowry et. al (1951), and by kit Dumas B.T. et. al (1971), Gornall A.G. et. al (1949) Rodkey F.L. (1965) and Verly H (1980) serum protein level obtained in normal condition of the mammals presented in Table 25.

In human (Homo sapiense) serum protein level mean valu ewere 7.20 ± 0.30 g/dl goat (capra Hircus) 6.30 ± 0.20 g/dl sheep ovis ovis, 5.60 ± 0.40 g/dl. Cow (Bos indicus) 7.10 ± 0.10 g/dl and in Dog (Canis familiaris) 6.20 ± 0.60 g/dl.

SERUM PROTEIN LEVEL IN NORMAL CONDITION OF THE MAMMALS

Mammalian (Species)	Serum Protein (g/dl)
Humen (Homo Sapiense)	7.20 ± 0.30
Goat (Capra Hircus)	6.30 ± 0.20
Sheep (Ovis aries)	5.60 ± 0.40
Cow (Bos Indicus)	7.10 ± 0.10
Dog (Canis Familiaris)	6.20 ± 0.60

Table No. 25 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ P < 0.05 (Significant difference)

++ P < 0.01 (Significant difference)

The Serum protein content were higher in Humen then other mammals. Taylor et al (1949) observed the range of serum prolein level in humen was 7.0 ± 0.4 g/dl.

The serum protein level content obtained were lowest in the sheep (ovis ovis). It we arranging the serum protein content mean of the mammals (Table 25) increasing order the data will be Humen > Cow > goat > dog > sheep.

➢ The serum protein level also observed in cold climatic condition of the mammals presented in the table 26. The data obtained in cold climatic condition have non-significant difference then normal condition of the mammals.

The mean of cold climatic data were Humen (Homo Sapiense) 7.20 ± 0.35 g/dl. Goat (Capra Hircus) 6.30 ± 0.28. g/dl. Sheep ovis ovis) 5.60 ± 0.47 g/dl. Cow (Bos indicus) 7.10 ± 0.15 g/dl and Dog (Canis familiaris) 6.20 ± 0.68.

The value obtain arranging in increasing order of serum protein level was the same as in normal condition.

➢ The serum protein level were observed in hot climatic condition of the mammals. Which is presented in the table 27. The data obtained in hot climatic condition have non significant different then normal condition of the mammals.

The Mean of hot climatic value were Humen (Homo sapiense) 7.10 ± 0.20 g/dl. goat (capra Hircus) 6.20 ± 0.60 g/dl., sheep (Ovis Ovis), 5.50 ± 0.70 g/dl., Cow (Bos indicus) 7.00 ± 0.20 g/dl., and Doy (Canis familiaris) 6.20 ± 0.50 g/dl., The value obtained arranging en increasing order of the serum protein were the same as in normal condition. Humen >Cow> goat > Dog > Sheep.

SERUM PROTEIN LEVEL IN COLD CLIMATE CONDITION OF THE MAMMALS

Mammalian (Species)	Serum protein level (g/dl)	
	Normal Values	Cold Climatic value
Humen (Homo Sapiense)	7.20 ± 0.3	7.20 ± 0.35
Goat (Capra Hircus)	6.30 ± 0.2	6.30 ± 0.28
Sheep (Ovis aries)	5.60 ± 0.4	5.60 ± 0.47
Cow (Bos Indicus)	7.10 ± 0.1	7.10 ± 0.15
Dog (Canis Familiaris)	6.20 ± 0.6	6.20 ± 0.68

Table No. 26 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

SERUM PROTEIN LEVEL IN HOT CLIMATE CONDITION OF THE MAMMALS

Mammalian (Species)	Serumprotein (g/dl)	
	Normal Value	Hot Climatic value
Humen (Homo Sapiense)	7.20 ± 0.3	7.10 ± 0.20
Goat (Capra Hircus)	6.30 ± 0.2	6.20 ± 0.60
Sheep (Ovis aries)	5.60 ± 0.4	5.50 ± 0.70
Cow (Bos Indicus)	7.10 ± 0.1	7.00 ± 0.20
Dog (Canis Familiaris)	6.20 ± 0.6	6.20 ± 0.50

Table No. 27 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

SERUM PROTEIN LEVEL IN DIFFERENT SEASION (DEFFERENT CLIMATIC CONDITION) OF THE MAMMALS

Mammalian (Species)	Blood Glucose level in (Mg/dl)		
	Mansoon[1]	Winter[2]	Summer[3]
Humen (Homo Sapiense)	7.20 ± 0.30	7.20 ± 0.35	7.10 ± 0.20
Goat (Capra Hircus)	6.30 ± 0.20	6.30 ± 0.28	6.20 ± 0.60
Sheep (Ovis aries)	5.60 ± 0.40	5.60± 0.47	5.50 ± 0.70
Cow (Bos Indicus)	7.10 ± 0.10	7.10 ± 0.25	7.00 ± 0.20
Dog (Canis Familiaris)	6.20 ± 0.60	6.20 ± 0.68	6.20 ± 0.50

Table No. 28 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

Serum protein level were observed in different climatic condition of the mammals.

Which is presented in table 28. The data obtained in different climatic condition have non Significant difference then normal condition of the mammals. There were non significant effect of season. In serum protein level on the mammal.

SERUM PROTEIN LEVEL IN DIFFERENT AGE GROUP OF THE MAMMALS :

Serum protein level also observed in different age group of the mammals which were presented in table 29-32 the serum protein level of age group child hood and old age group child hood and old age in each mammal have non significant difference.

Yet age group adult in each mammals Humen, goat, sheep. Cow and Dog) and Dog) have high significant difference (PLO.05). Orlandini et al (1955) observed the mean of serum protein level 6.2 ± 0.5 in child hood of humen.

The mean value of total serum protein in adult age of mammals obtain Humen (Homo-sapience) 7.20 ± 0.30 g/dl. Goat (Capra-Hircus) 6.30 ± 0.20 g/dl. sheep covis over) 5.60 ± 0.40 g/dl. Cow (Bos in dicus) 7.10 ± 0.10 g/dl. and in Dog (Canis familiaris) 6.20 ± 0.60 g/dl.

Increase total serum protein levels with age of mammals indicated linear rises along with functional age of mammals which may be attributed to physiological factors (tumble son et al 1973, Gaikwad et al 1992.

➢ Serum protein level observed in protein rich diet of the mammals. Which were presented in (table 33) the total protein

SERUM PROTEIN LEVEL IN CHILD AGE GROUP OF THE MAMMALS

Mammalian (Species)	Age group	Serum protein level (g/dl.)
Humen (Homo Sapiense)	1 - 5 Years	6.10 ± 0.50
Goat (Capra Hircus)	3 months - 1 years	6.026 ± 0.04
Sheep (Ovis aries)	3 months - 1 years	5.16 ± 0.24
Cow (Bos Indicus)	3 months - 2 years	6.80 ± 0.18
Dog (Canis Familiaris)	3 months - 1 years	5.90 ± 0.10

Table No. 29 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

SERUM PROTEIN LEVEL IN ADULT AGE GROUP OF THE MAMMALS

Mammalian (Species)	Age group	Serum Protein (g/dl.)
Humen (Homo Sapiense)	21 - 30 Years	7.20 ± 0.30
Goat (Capra Hircus)	2 - 5 years	6.30 ± 0.20
Sheep (Ovis aries)	2 - 5 years	5.60 ± 0.40
Cow (Bos Indicus)	3 - 8 years	7.60 ± 0.10
Dog (Canis Familiaris)	2 - 5 years	6.20 ± 0.60

Table No. 30 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

SERUM PROTEIN LEVEL IN OLD AGE GROUP OF THE MAMMALS

Mammalian (Species)	Age group	Serum Protein (g/dl.)
Humen (Homo Sapiense)	above 60 years	7.12 ± 0.06
Goat (Capra Hircus)	above 5 years	6.12 ± 0.08
Sheep (Ovis aries)	above 5 years	5.26 ± 0.34
Cow (Bos Indicus)	above 8 years	6.86 ± 0.15
Dog (Canis Familiaris)	above 5 years	5.92 ± 0.23

Table No. 31 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ P < 0.05 (Significant difference)

++ P < 0.01 (Significant difference)

SERUM PROTEIN LEVEL IN DIFFERENT AGE GROUPS OF THE MAMMALS

Mammalian Species	Age groups	Serum Protein Level (g/dl)
Humen (Homo Sapiense)	1 - 5 Years	6.10 ± 0.50
	21 - 30 Years	7.20 ± 0.30+
	Above 60 Years	7.12 ± 0.06
Goat (Capra Hircus)	3 months 1 years	6.02 ± 0.04
	2 to 5 years	6.30 ± 0.20+
	above 5 years	6.12 ± 0.08
Sheep (Ovis aries)	3 months to 1 years	5.16 ± 0.24
	2 - 5 years	5.60 ± 0.40+
	above 5 years	5.26 ± 0.34
Cow (Bos Indicus)	6 month to 2 years	6.80 ± 0.18
	5 - 8 years	7.10 ± 0.10+
	above 8 years	6.86 ± 0.15
Dog (Canis Familiaris)	3 months to 1 years	5.90 ± 0.10
	2 to 5 years	6.20 ± 0.60+
	above 5 years	5.92 ± 0.23

Table No. 32 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

SERUM PROTEIN LEVEL IN PROTEIN RICH DIET OF THE MAMMALS

Mammalian (Species)	Serumprotein (g/dl)	
	Normal Values	After Protein rich diet value
Humen (Homo Sapiense)	7.20 ± 0.3	7.20 ± 0.50
Goat (Capra Hircus)	6.30 ± 0.2	6.30 ± 0.70
Sheep (Ovis aries)	5.60 ± 0.4	5.60 ± 0.60
Cow (Bos Indicus)	7.10 ± 0.1	7.10 ± 0.40
Dog (Canis Familiaris)	6.20 ± 0.6	6.20 ± 0.80

Table No. 33 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ P < 0.05 (Significant difference)

++ P < 0.01 (Significant difference)

SERUM PROTEIN LEVEL IN PROTEIN GRAZING MAMMALS

Mammalian (Species)	Serumprotein (g/dl)	
	Normal Value	Value in grazing mammals
Goat (Capra Hircus)	6.30 ± 0.20	5.20 ± 0.50+
Sheep (Ovis aries)	5.60 ± 0.40	4.70 ± 0.30+
Cow (Bos Indicus)	7.10 ± 0.10	6.00 ± 0.20+

Table No. 34 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

SERUM PROTEIN LEVEL IN LACTATING OF THE MAMMALS

Mammalian (Species)	Serum Protein (g/dl)		
	Goat	Sheep	Cow
Humen (Homo Sapiense)	6.30 ± 0.20	5.60 ± 0.40	7.10 ± 0.10
Goat (Capra Hircus)	6.45 ± 0.50	5.70 ± 0.30	7.40 ± 0.20
Sheep (Ovis aries)	6.55 ± 0.45	5.83 ± 0.72	7.60 ± 0.40
Cow (Bos Indicus)	6.20 ± 0.30	5.40 ± 0.60	7.20 ± 0.30
Dog (Canis Familiaris)	6.90 ± 0.10	6.10 ± 0.90	7.90 ± 0.20

Table No. 35 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

concentration in serum did not differ significantly the serum protein remains constant through out 60 days of protein feeding.

The daily profile observed in those protein was indeed surprising when compared with published data. That indicate no variation during the 24 hour period (Rowlands 1980, manston et al. 1981, clement et al 1991).

- Serum protein level also observed in graging mammals. which ks presented in (table 34).

The serum protein level highly significant deference (PL 0.05) decreases then normal condition of the mammals there was reduction in total protein due to national deficiency the report of the present study indicated that higher prevalence of mammals suggested the possibility that mammals of temperate type sweat profusely and lose saliva and mucus from the month resulting of minerals in grazing mammals.

- Serum protein level in lactating, which was presented in (Table -35) concentration of serum protein, was higher (PL 0.01) at 90 day post padding. Then other periods because of added requirement of full term fetus in former and energy drain from body in peak lactation in the later situation,

Serum albumin level in different mammals :

The serum albumin level were observed in different mammals.

Serum separated from the samples collected was stored in refrigerator until analysis were completed. mostly fresh separate serum was used for analysis of albumin.

The method followed by modified Buret and dumas (1971) through kit.

SERUM ALBUMIN LEVEL IN NORMAL CONDITION OF THE MAMMALS

Mammalian (Species)	Serum Albumin (g/dl)
Humen (Homo Sapiense)	4.50 ± 0.60
Goat (Capra Hircus)	3.60 ± 0.30
Sheep (Ovis aries)	2.30 ± 0.40
Cow (Bos Indicus)	3.42 ± 0.58
Dog (Canis Familiaris)	3.20 ± 0.25

Table No. 36 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

SERUM ALBUMIN LEVEL IN COLD CLIMATIC CONDITION OF THE MAMMALS

Mammalian Species	SerumAlbumin (g/dl)	
	Normal Values	Cold Climatic values
Humen (Homo Sapiense)	4.50 ± 0.60	4.63 ± 0.37
Goat (Capra Hircus)	3.60 ± 0.30	3.72 ± 0.22
Sheep (Ovis aries)	2.30 ± 0.40	2.50 ± 0.50
Cow (Bos Indicus)	3.42 ± 0.58	3.53 ± 0.28
Dog (Canis Familiaris)	3.20 ± 0.25	3.30 ± 0.32

Table No. 37 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ P < 0.05 (Significant difference)

++ P < 0.01 (Significant difference)

higher in human and lower in sheep. If we arranging it their values in increasing order will be humen > goat > Cow > Dog > Sheep.

The serum albumin level also observed of the that climatic condition mammals. Which was presented in table-38.

Highly significant different ($P<0.05$) observed in hot climatic condition then normal condition.

Dehydration is one condition in which the increase in total protein is due to increase in both albumin and globulin fraction because of haemoconcentration.

In this case the albumin/globulin retio remains altered.

Except dehydration an increase in albumin very rarely occurs. The serum albumin level also observed in different climatic condition (seasion) which was presented in (Table-39) According to the observation in different climatic condition (Table-39) result shows that highly significant difference ($P <0.01$) was observed in albumin level between monsoon (normal condition) and cold climatic condition (winter) and a significant deference ($P < 0.05$) increase was found in normal condition (monsoon)then hot climatic condition (summer). If we arranging their mean values in increasing order will be same as normal condition.

Human (Homo sapiense) > Goat (Capra Hircus) > Cow (Bos indicus) > Dog (Canis familiaris) > Sheep (ovis ovis)

These variation in serum albumin level were due to the physiological and environmental condition on the mammals.

Serum albumin level also observed in deferent age group of the mammals, which was presented in (Table-40-43).

Serum albumin level was significantly ($P <0.05$) lowest in old age group comparision to childage and adult age in all the mammals (Humen, goat, sheep cow & Dog). In adult age serum albumin level were higher ($P <0.01$) then childage in all cases.

SERUM ALBUMIN LEVEL IN HOT CLIMATIC CONDITION OF MAMMALS

Mammalian (Species)	Serum protein (g/dl)	
	Normal Values	Hot Climatic values
Humen (Homo Sapiense)	4.50 ± 0.60	4.72 ± 0.37
Goat (Capra Hircus)	3.60 ± 0.30	3.73 ± 0.27
Sheep (Ovis aries)	2.30 ± 0.40	2.65 ± 0.55
Cow (Bos Indicus)	3.42 ± 0.58	3.65 ± 0.47
Dog (Canis Familiaris)	3.20 ± 0.25	3.58 ± 0.35

Table No. 38 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

SERUM ALBUMIN LEVEL IN DIFFERENT SEASION (DEFFERENT CLIMATIC CONDITION) OF THE MAMMALS

Mammalian (Species)	Blood Glucose level in (Mg/dl)		
	Mansoon[1]	Winter[2]	Summer[3]
Humen (Homo Sapiense)	4.50 ± 0.60	4.63 ± 0.37	4.72 ± 0.68
Goat (Capra Hircus)	3.60 ± 0.30	3.72 ± 0.27	3.78 ± 0.30
Sheep (Ovis aries)	2.30 ± 0.40	3.45 ± 0.50	2.50 ± 0.55
Cow (Bos Indicus)	3.42 ± 0.58	3.53 ± 0.28	3.64 ± 0.47
Dog (Canis Familiaris)	3.20 ± 0.25	3.30 ± 0.32	3.58 ± 0.35

Table No. 39 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

SERUM ALBUMIN LEVEL IN CHILD AGE GROUP OF THE MAMMALS

Mammalian (Species)	Age group	Serum Albumin level (g/dl.)
Humen (Homo Sapiense)	1 - 5 Years	4.16 ± 0.44
Goat (Capra Hircus)	3 months - 1 years	3.23 ± 0.57
Sheep (Ovis aries)	3 months - 1 years	2.13 ± 0.52
Cow (Bos Indicus)	3 months - 2 years	3.27 ± 0.53
Dog (Canis Familiaris)	3 months - 1 years	3.08 ± 0.11

Table No. 40 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P \leq 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

SERUM ALBUMIN LEVEL IN ADULT AGE GROUP OF THE MAMMALS

Mammalian (Species)	Age group	Serum Albumin (g/dl.)
Humen (Homo Sapiense)	21 - 30 Years	4.50 ± 0.60
Goat (Capra Hircus)	2 - 5 years	3.60 ± 0.30
Sheep (Ovis aries)	2 - 5 years	2.30 ± 0.04
Cow (Bos Indicus)	3 - 8 years	3.42 ± 0.58
Dog (Canis Familiaris)	2 - 5 years	3.20 ± 0.25

Table No. 41 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ P < 0.05 (Significant difference)

++ P < 0.01 (Significant difference)

SERUM ALBUMIN LEVEL IN OLD AGE GROUP OF THE MAMMALS

Mammalian (Species)	Age group	Serum Albumin level (g/dl.)
Humen (Homo Sapiense)	above 60 years	3.82 ± 0.24
Goat (Capra Hircus)	above 5 years	3.01 ± 0.18
Sheep (Ovis aries)	above 5 years	2.01 ± 0.21
Cow (Bos Indicus)	above 8 years	3.08 ± 0.22
Dog (Canis Familiaris)	above 5 years	3.06 ± 0.21

Table No. 42 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ P < 0.05 (Significant difference)

++ P < 0.01 (Significant difference)

SERUM ALBUMIN LEVEL IN DIFFERENT AGE GROUPS OF THE MAMMALS

Mammalian Species	Age groups	Serum Alubumin Level (g/dl)
Humen (Homo Sapiense)	1 - 5 Years	4.16 ± 0.44
	21 - 30 Years	4.50 ± 0.60+
	Above 60 Years	3.82 ± 0.24
Goat (Capra Hircus)	3 months 1 years	3.23 ± 0.57
	2 to 5 years	3.60 ± 0.30+
	above 5 years	3.01 ± 0.18
Sheep (Ovis aries)	3 months to 1 years	2.13 ± 0.52
	2 - 5 years	2.30 ± 0.04+
	above 5 years	2.01 ± 0.21
Cow (Bos Indicus)	6 month to 2 years	3.27 ± 0.53
	5 - 8 years	3.42 ± 0.58+
	above 8 years	3.08 ± 0.22
Dog (Canis Familiaris)	3 months to 1 years	3.08 ± 0.11
	2 to 5 years	3.20 ± 0.25+
	above 5 years	3.06 ± 0.21

Table No. 43 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

This change appears to by physiological one when considering total serum protein and serum albumin levels. Semilar finding were reported by Gaikwad et al. 1992.

Serum albumin level also observed in ,protein rich diet of the mammals which is presented in Table-44.

The serum albumin concentration in serum remained constant through 60 days of protein rich diet feeding The events that take place during and immediately after the act of eating in particular the passage of plasma fluid to extracellular space and then to the digestive tract (Bailey. 1961: Stacy and Warner (1966) cited in Christopherson and Webster (1972) and the increase of osmotic pressure (Stacy and Warner (1966) cited in Christopherson and Webster (1972): Ternouth (1968) could possibly explain a parallel movement of albumin from intravascular to extravascular space, to prevent abrupt and dangerous variations of oncotic pressure. Since the permeability of vascular walls to albumin is low, movement of albumin probably occurs mainly across the large pores of the liver sinusoids. The increase in arterial pressure and blood flow in these vessels during and after a meal (lomax and baird 1983; Mineo et al. 1991) lead to a transudation to the lymph of significant amount of fluid with 80 to 90% protein plasma concentration (Vernon and Peaker 1983 : Guyton 1986). In addtion, some authors indicate that large amounts of plasma protein, in particular albumin because of its small size in comparison with Globulin, pass to the extravacular space in a 24-h period (Smith and Hamlin 1977; Harrison 1985; Berne and Levy 1988) in order to counterbalance oncotic pressure variations (Harrison 1985).

Both the negative correlation between albumin serum concentration and that of albumin (Payne and Payne 1987), and its

SERUM ALBUMIN LEVEL IN PROTEIN RICH DIET OF THE MAMMALS

Mammalian (Species)	SerumAlbumin (g/dl)	
	Normal Value	After protein diet value
Humen (Homo Sapiense)	4.50 ± 0.60	4.55 ± 0.55
Goat (Capra Hircus)	3.60 ± 0.30	3.62 ± 0.28
Sheep (Ovis aries)	2.30 ± 0.40	2.36 ± 0.25
Cow (Bos Indicus)	3.42 ± 0.58	3.53 ± 0.47
Dog (Canis Familiaris)	3.20 ± 0.25	3.31 ± 0.45

Table No. 44 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

SERUM ALBUMIN LEVEL IN LACTATING PERIOD OF THE MAMMALS

Lactating Days	Goat	Sheep	Cow
Day of Kidding	3.60 ± 0.30	2.30 ± 0.40	3.42 ± 0.58
3rd day of lactating	3.49 ± 0.12	2.18 ± 0.22	3.23 ± 0.37
7th day of lactating	3.38 ± 0.10	2.08 ± 0.13	3.10 ± 0.21
30th day of lactating	3.10 ± 0.13	2.00 ± 0.11	2.90 ± 0.23
90th day of lactating	2.85 ± 0.16	1.80 ± 0.20	2.76 ± 0.10

Table No. 45 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

secondary role on oncotic pressure seem to justify these daily profiles as compensations for albumin variations.

Serum albumin level also observed dactating period of the mammals, which is presented in Table-45.

Concentration of serum albumin where higher (P<0.01) at 90th day post kidding then other periods 3rd day, 7th day and 30th days of lactation. Because of added requirement of full terms fetus in former and energy drain from the body in peak lactation in the later situation. In general the normal values of blood metabolites observed in the study indicated that concentrate supplementation were fed adequately.

Blood Urea level in different mammals :

The blood urea level were observed in different mammals. The blood was collected in vial with lithium oxalate (1.5 mg./ml) as anti coagulant which was very useful for blood urea nitrogen and is an enzyme poison and inhibits the enzymatic reaction. The method followed by Rashid Ali (1992) which was modified method of coulombe et al (1963) crocker C.L. (1967), fearon W.R. (1939), Hunni ghake et al (1966), marsh at it (1965), Teitz N.W. (1983) and through kit levinson. S.S. (1978), and liquang C.T. (1987).

Blood urea content obtained in normal condition of the mammals presented in (Table. 46).

Significant difference (P <. 0.05) mean value of blood urea level observed different mammals.

In Human (Homosapiense) 26.20 +_ 2.30 mg/dl ., sheep (ovis ovis) 13.08 +_ 1.12 mg/dl. Cow (Bos indicus) 11.25 +_ 1.75 mg/dl and in Dog (Canis familiars) 14.01 +_ 1.08 in the human the blood urea content were higher then other mammals such as goad sheep cow and Dog.

BLOOD UREA LEVEL IN NORMAL CONDITION OF THE MAMMALS

Mammalian (Species)	Blood Urea Level (mg/100 ml)
Humen (Homo Sapiense)	26.20 ± 2.30
Goat (Capra Hircus)	20.50 ± 1.30
Sheep (Ovis aries)	13.08 ± 1.12
Cow (Bos Indicus)	11.25 ± 1.75
Dog (Canis Familiaris)	14.01 ± 1.08

Table No. 46 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

Jellinek & Looney (1943) observed the blood urea range in the human mean value 32.1 +_ 3.4 mg/dl. reported the blood urea metrogen content obtained were (table) lowest in cow (Bos endicas) The data shows that the human blood contain lowest urea level.

Urea is the waste product of protein metabolsm and is formed mainly from the amino acids in the liver. So blood urea concentration is influenced by the amount of protein taken in the diet. In tends to be on lower side of the normal range in people on low protein diet. Urea from the blood is filtered by glomeruli which is governed by the net filtration pressure derived from the hydrostatic pressure of the blood and by the amount of blood flowing through the kidney. So any condition, renal or extra renal, which affects the net filtration pressure will reduce the glomerular filtration rate and blood urea will be found raised.

➢ The blood urea level also observed in cold climatic condition of the mammals which is presented in (Table-47).

Non significant different were found in cold climatic condition in all the mammals such as Human (Homo sapiense), goal (Capra Hircus) sheep (ovis ovis), Cow (Bos indices) and Dog (Canis familearis).

The data obtained in cold climate condition mean value were Human (28.01+_ 1.19) mg/dl, goal (22.03+_ 1.27) mg/dl, sheep (15.10+_1.30) mg/dl, Cow (13.20 +_ 1.15) mg/dl. It we arranging according to blood urea concentration in increasing order Human > Goat < sheep > cow.

BLOOD UREA LEVEL IN COLD CLIMATIC CONDITION OF THE MAMMALS

Mammalian (Species)	Blood urea (mg/100 ml)	
	Normal Values	Cold Climatic value
Humen (Homo Sapiense)	26.20 ± 2.30	28.01 ± 1.19
Goat (Capra Hircus)	20.50 ± 1.30	22.03 ± 1.27
Sheep (Ovis aries)	13.08 ± 1.12	15.10 ± 1.30
Cow (Bos Indicus)	11.25 ± 1.75	13.20 ± 1.60
Dog (Canis Familiaris)	14.01 ± 1.08	16.05 ± 1.15

Table No. 47 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

➢ The blood urea level observed in hot climatic condition of the mammals which presented in (Table-48).

Non significant different were found in hot climatic condition in all the experimented mammals such as Human sheep (Ovis Ovis) cow (Bos endicus) and Dog (canis familiars) The data obtained in hot climate condition mean value were Human (30+_ 24+_ 1.26), mg/dl, Goat (24.11 +_ 1.19) mg/dl, sheep (ovis ovis) (17.27 +_ 1.30) mg/dl, cow 15.34 +_ 1.26) mg/dl, and Dog (16.14 +_ 1.23) It we arrange it according to blood urea concentration increasing order data will se same as in cold climatic or normal condition of the mammals.

➢ Blood urea also observed in different climatic condition (seasion) which is presented in (Table -49).

Non significant difference observed in different climatic condition in all the experimented mammals such as Human (Homo sapeince) goat (capra Hircus) sheep (ovis ovis) Cow (Bos endicus) & Dog (camis familiars) If we arranging their values which obtained in table monsoon, (Normal condition) winter (cold climatic condition) and summer (hol climatic condition) increasing order there were non significant order there were non significant effect of seasion in blood urea concentration increasing order will se same as in normal, cold and hot climatic condition in all the mammals such as Human > goat > Dog > sheep > Cow.

➢ Blood urea also observed in different age group of the mammals (Humen, Goat, Sheep, Cow & Dog).

Which is presented in (Table 50-53).

None significant difference observed in different age group

BLOOD UREA LEVEL IN DIFFERENT CLIMATIC CONDITION (SEASION) OF THE MAMMALS

Mammalian (Species)	Blood Glucose level in (Mg/dl)		
	Mansoon[1]	Winter[2]	Summer[3]
Humen (Homo Sapiense)	26.20 ± 2.30	28.01 ± 1.19	30.24 ± 1.26[+]
Goat (Capra Hircus)	20.50 ± 1.30	22.03 ± 1.27	24.11 ± 1.19[+]
Sheep (Ovis aries)	13.08 ± 1.12	15.10 ± 1.30	17.27 ± 1.30[+]
Cow (Bos Indicus)	11.25 ± 1.75	13.20 ± 1.60	15.34 ± 1.26[+]
Dog (Canis Familiaris)	14.01 ± 1.08	16.05 ± 1.15	16.14 ± 1.23[+]

Table No. 49 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

BLOOD UREA LEVEL IN CHILD AGE GROUP OF THE MAMMALS

Mammalian (Species)	Age group	Blood urea level (mg/dl.)
Humen (Homo Sapiense)	1 - 5 Years	25.12 ± 1.08
Goat (Capra Hircus)	3 months - 1 years	20.32 ± 1.12
Sheep (Ovis aries)	3 months - 1 years	12.86 ± 1.04
Cow (Bos Indicus)	3 months - 2 years	11.10 ± 1.06
Dog (Canis Familiaris)	3 months - 1 years	13.92 ± 1.03

Table No. 50 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

BLOOD UREA LEVEL IN ADULT AGE GROUP OF THE MAMMALS

Mammalian (Species)	Age group	Blood Urea Level (mg/dl.)
Humen (Homo Sapiense)	21 - 30 Years	26.20 ± 2.30
Goat (Capra Hircus)	2 - 5 years	20.50 ± 1.30
Sheep (Ovis aries)	2 - 5 years	13.08 ± 1.12
Cow (Bos Indicus)	3 - 8 years	11.25 ± 1.75
Dog (Canis Familiaris)	2 - 5 years	11.25 ± 1.08

Table No. 51 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

BLOOD UREA LEVEL IN OLD AGE GROUP OF THE MAMMALS

Mammalian (Species)	Age group	Blood Urea Level (mg/dl.)
Humen (Homo Sapiense)	above 60 years	26.24 ± 2.25
Goat (Capra Hircus)	above 5 years	20.64 ± 1.44
Sheep (Ovis aries)	above 5 years	13.28 ± 1.05
Cow (Bos Indicus)	above 8 years	11.32 ± 1.78
Dog (Canis Familiaris)	above 5 years	14.21 ± 1.08

Table No. 52 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

BLOOD UREA LEVEL IN DIFFERENT AGE GROUPS OF THE MAMMALS

Mammalian Species	Age groups	Blood Urea Level (mg/dl)
Humen (Homo Sapiense)	1 - 5 Years	25.12 ± 1.08
	21 - 30 Years	26.20 ± 2.30
	Above 60 Years	26.24 ± 2.25
Goat (Capra Hircus)	3 months 1 years	20.32 ± 1.12
	2 to 5 years	20.50 ± 1.30
	above 5 years	20.64 ± 1.44
Sheep (Ovis aries)	3 months to 1 years	12.86 ± 1.04
	2 - 5 years	13.08 ± 1.12
	above 5 years	13.28 ± 1.05
Cow (Bos Indicus)	6 month to 2 years	11.10 ± 1.06
	5 - 8 years	11.25 ± 1.75
	above 8 years	11.32 ± 1.78
Dog (Canis Familiaris)	3 months to 1 years	13.92 ± 1.03
	2 to 5 years	14.01 ± 1.08
	above 5 years	14.21 ± 1.04

Table No. 53 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

BLOOD UREA LEVEL IN HOT CLIMATIC CONDITION OF THE MAMMALS

Mammalian (Species)	Blood urea (mg/100 ml)	
	Normal Value	Hot Climatic value
Humen (Homo Sapiense)	26.20 ± 2.30	30.24 ± 1.26+
Goat (Capra Hircus)	20.50 ± 1.30	24.11 ± 1.19+
Sheep (Ovis aries)	13.08 ± 1.12	17.27 ± 1.30+
Cow (Bos Indicus)	11.25 ± 1.75	15.34 ± 1.26+
Dog (Canis Familiaris)	14.01 ± 1.08	16.14 ± 1.23+

Table No. 48 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

childhood, Adult and old age) of the experimented mammals. such as Human (Homo sapeinse), goat (Capra hircus), sheep (ovis ovis) Cow (Bos indicus) and Dog (canis familearer) If we arranging their values which obtained in (table) childhood Adult and old age in increasing order.

There mean value is blood urea concentration increasing order will be Human> Goat> Dog >sheep > Cow. Similar finding were reported by ShriKhande et al 1999. in case of cow.

Blood urea level also observed in protein rich diet treated 60 days in all experimented mammals (Human) (Homo sepiense) goat (capra hircus) sheep (ovis ovis), Cow (Bos indicus) Dog (Canis familiaris) which is presented in (Table-54).

Significant difference ($P < 0.01$) increases observed in each case of mammals.

Higher concentration of blood urea level indicated that protein rich diet increases the blood urea each mammals.

The protein rich diet were recommended and calculated quantities periods of 60 days According to data obtained Table 38.

Human (29.10 ± 1.25) mg/dl. Goat (22.73 ± 1.27) mg/dl. Sheep (16.20 ± 1.08) mgfdl. Cow (14.07 ± 1.10) mg/dl. and Dog (17.03 ± 1.02) mg/dl.

A rise in blood urea level indicated that there was a possibility of renal tissue damage since elevated blood urea levels were reported. in renal necrosis, Kasen et al 1985 Senani et al 1994.

Urea were characterised by a gradual increase up to maximum value ($P < 005$) after the meal, then declining to basal values the, increase after the meal was also evident, but basal urea

BLOOD UREA LEVEL IN PROTEIN RICH DIET OF THE MAMMALS

Mammalian (Species)	Blood urea (mg/100 ml)	
	Normal Values	After protein rich diet values
Humen (Homo Sapiense)	26.20 ± 2.30	29.10 ± 1.25
Goat (Capra Hircus)	20.50 ± 1.30	22.73 ± 1.27
Sheep (Ovis aries)	13.08 ± 1.12	16.20 ± 1.08
Cow (Bos Indicus)	11.25 ± 1.75	14.07 ± 1.10
Dog (Canis Familiaris)	14.01 ± 1.08	17.03 ± 1.02

Table No. 54 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

concentration before and after that period were higher ($P < 0.05$) than those observed in the resulting in a greater overall mean In value this pattern is relatively well known in ruminants, particularly the increase after the meal (Manston et al. 1981; cisse et al. 1991; Clement et .al. 1991).

➢ Blood urea levels also observed in. Fat diet of the mammals. Which was presented in (Table-55).

Blood urea concentration measured after 7 days of fat rich in the mammals Human (Homo sapiense), Goat (Capra Hircus), Sheep (Ovis Ovis), Cow (Bos indicus) and in Dog (canis familiaris) did not differ significantly from the normal value, mean of fat rich values were Huaman (26.83 + 2.12) mg/dl., Goat (21.10 ± 1.39) mg/dl, Sheep (13.68 ± 1.15) mg/dl, and Dog (14.68 ± 110) mg/dl.

If we arranging the mean values in increasing order it will se Human Goat> Dog> Sheep> Cow.

➢ The blood urea level also observed in grazing mammals. Which is presented in (Table -56).

Highly significant ($P < 0.01$) difference increase all grazing mammals such as goat (Capra Hircus) sheep (ovis ovis) and cow. (Bos indicus) were observed the. mean of blood urea concentration in all mammals were Goat (27.08 ± 0.18) mg/dl , Sheep (20.01 ± 0.13) mg/dl, and cow (22.12 ± 1.8) mg/dl.

The increasing value of these mammals were goat> cow> sheep.

In mammals undernutrition the mobilisation and utilisation of amino acids for glucose and (oxaloacetate production are increased, which is reflected in high Urea values (Cocimano and Leng 1967;

BLOOD UREA LEVEL IN FAT RICH DIET OF THE MAMMALS

Mammalian (Species)	Blood urea (mg/100 ml)	
	Normal Value	After Fat rich diet value
Humen (Homo Sapiense)	26.20 ± 2.30	26.83 ± 2.12
Goat (Capra Hircus)	20.50 ± 1.30	21.10 ± 1.39
Sheep (Ovis aries)	13.08 ± 1.12	13.68 ± 1.15
Cow (Bos Indicus)	11.25 ± 1.75	11.75 ± 1.85
Dog (Canis Familiaris)	14.01 ± 1.08	14.68 ± 1.10

Table No. 55 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

BLOOD UREA LEVEL IN GRAZING MAMMALS

Mammalian (Species)	Blood urea (mg/100 ml)	
	Normal Value	Value in Grazing Mammals
Goat (Capra Hircus)	20.50 ± 1.30	27.08 ± 0.18
Sheep (Ovis aries)	13.08 ± 1.12	20.01 ± 0.13
Cow (Bos Indicus)	11.25 ± 1.75	22.12 ± 1.08

Table No. 56 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

Gall et al. 1993) Difference in overall means may also be explained by the level of protein, nitrogen and energy in experimental diets. Greater values were probably not observed because urea excretion increases linearly above urea concentrations. (Cocimano and Leng 1967).

Serum creatinine in different mammals :

The serum creatinine level wee observed in different mammals. Serum separated from the samples collected was stored in refrigerator until analysis were completed was stroed in refrigrator until analysis were completed mostly fresh seprate serum was used for analysis of creatinine.

The method was followed by modified Version of rod et. al (1948) by kit R. Helger et. al (1971).

Bartel H. et al. (1972) and allmann R et. al (1976).

The serum creatinine level were measured by colorimetrically at 540 nm and by kit chemistry autonalizer RA-50 used for reading.

Serum ereatinine level obtained in normal condition of the mmals presented in (Table -57).

A significant ($P < 0.05$) difference mean value were observed in different experimented mammals as (Humen (Homo sapiense) Goat (Capra Hircus) Sheep (ovis ovis) Cow (Bos endicus) & Dog (canis formiliaris) the mean of obtained value were -

Humen (1.03 ± 0.17) mg/dl, Goat (1.31 ± 0.05) mg/dl Sheep (1.42 ± 0.15) mg/dl. Cow (1.20 ± 0.12) mg/dl & in Dog (1.32 ± 0.03) mg/dl.

SERUM CREATININE LEVEL IN NORMAL CONDITION OF THE MAMMALS

Mammalian (Species)	**Serum Cretinine (mg/dl)**
Humen (Homo Sapiense)	1.03 ± 0.17
Goat (Capra Hircus)	1.31 ± 0.05
Sheep (Ovis aries)	1.42 ± 0.15
Cow (Bos Indicus)	1.20 ± 0.12
Dog (Canis Familiaris)	1.32 ± 0.03

Table No. 57 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

If we arranging the mean value obtained in encreasing order will be - Sheep > Goat > Dog > Cow > Humen.

Morgan et al (1956) reported in Humen Serm creatinine mean value (1.27 ± 0.30) mg/dl.

Creatinine is an anhydride of creatine and is formed largely in muscles by the irreversible nonezymatic removal of water from creatine. There is evidence that the tubules can excrete a cerain amount of creatinine in man at any rate at raised blood creatinine level, so this may contribute to its removal from the blood. Moreover, the pre-renal factors which increase blood urea have little influence on the serum creatinine level. Due to these reasons creatinine may be normal when appreciable increase in blood urea has occured.

➢ Serum creatinine level also observed in protein rich diet of the mammals. Which was presented in Table-58) Serum creatinine concentration decreased ($P < 0.05$) after 60 days of feeding.

The protein rich diet was recommended and calculated quantities.

The mean value of creatinine concentrations were Humen (Homo sapiense) 1.00 ± 0.30 Goat (Capra Hircus) 1.32 ± 0.16 mg/dl. Cow (Bos endicus) 1.10 ± 0.15 mg/dl. and in Dog (eanis familearis) 1.20 ± 0.50 mg/dl. If we arraging it according to the mean value in increasing order will be Sheep > Goat > Dog > Humen > Cow.

The most relevant events in serum creatinne evolution were the occurrence of maximum values after the intake of food and the decrease after the meal ($P < 0.05$). The decrease could possibly be explained by a reduction in the rate of creatinne synthesis from creatine because the inevitable high insulinemia, and the availability

SERUM CREATININE LEVEL IN PROTEIN RICH DIET OF THE MAMMALS

Mammalian (Species)	Serum Creatinine (mg/dl)	
	Normal Value	After protein rich diet value
Humen (Homo Sapiense)	1.03 ± 0.17	1.00 ± 0.30
Goat (Capra Hircus)	1.31 ± 0.05	1.21 ± 0.09
Sheep (Ovis aries)	1.42 ± 0.15	1.32 ± 0.16
Cow (Bos Indicus)	1.20 ± 0.12	1.10 ± 0.15
Dog (Canis Familiaris)	1.32 ± 0.03	1.20 ± 0.05

Table No. 58 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

of amino acids promote protein synthesis. After this anabolic period, the oreientation of overall metabolism will probably have changed to favouring catabolic pathways, thus the protein degradation and the consequent synthesis of creatinne. Although not statistically significant ($P > 0.05$), overall means suggest an inverse relationship of creatinne with nutritional level.

Serum Cholesterol level in different Mammals :

The serum cholesterol level were observed in different mammals. Serum separated from the samples collected was stored in refrigerator until analysis were completed. Mostly fresh separated serum was used for analysis of serum cholesterol. The method followed by Zlatkis A. et. al(1953) and Allain C.C. et. al (1974) (By kit). Serum cholesterol level were measured by colorimetrically at 625 nm and by kit through chemistry autoanalyzer RA - 50used for reading.

- Serum Cholesterol level were obtained in normal condition of the mammals. Which was presented in (Table-59).

Significant difference ($P < 0.05$) were obsered in mean value of the experimented mammals.(Humun - Homo sapcense, Goat - Capra Hircus, Sheep - ovis ovis, Cow - Bos indicus & Dog. - Caris familiaris).

The mean values of serum cholesterol in the mammals were - Humen (178.03 ± 32.02) mg/dl. Goat (105.01 ± 15.04) mg/dl. Sheep (63.02 ± 5.07) mg/dl, Cow (82.06 ± 13.03) mg/dl. & Dog (172.04 ± 36.0) mg/dl.

SERUM CHOLESTROL LEVEL IN NORMAL CONDITION OF THE MAMMALS

Mammalian (Species)	**Serum Cholestrol (mg/dl)**
Humen (Homo Sapiense)	178.03 ± 32.02
Goat (Capra Hircus)	105.01 ± 15.04
Sheep (Ovis aries)	63.02 ± 5.07
Cow (Bos Indicus)	82.06 ± 13.03
Dog (Canis Familiaris)	172.04 ± 36.01

Table No. 59 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

In thc Humen serum cholesterol mean value were higher then after mammals. Keys et. al (1950) observed the range of serum cholesterol mean value in humen (174 ± 32) mg/dl.

The Serum Cholesterol content obtained were lowest in the sheep (ovis ovis) Table.....

The data shows that humen required more serum cholesteral content then other mammals.

Cholesterol is undoubtedly the most publicized lipid in nature, because of the strong correlation between levels of cholesterol in the blood and the incidence of the cardiovascular system in humans. It is not only an important component of some cell membranes and of plasma lipoproteins but also the precursor of many other biologically important steroids, such as bile acids and various steroid hormones. It is the principals sterol of hither animals and is especially abundant in nerve tissues and in gallstones. In occurs either free or as fatty esters in all animal cells. It was first isolated in 1784, from human gallstones which consist almost entirely of cholesterol and hence so named (cholesterol literally means 'solid alcohol from bile'.

The great clinical significance of cholesterol level is attached to atherosclerosis and coronary heart disease. It is believed that persons having cholesterol level above 200 mg/100 ml are more susceptible to coronary heart disease. Patients with arterial disease can have any one of the following abnormalities of lipids level (Havel and Carlson, 1962).

- The serum cholesterol level also observed in cold climatic condition of the mammals. Which were presented in (Table-60).

SERUM CHOLESTEROL LEVEL IN COLD CLIMATIC CONDITION OF THE MAMMALS

Mammalian (Species)	Serum cholesterol level (mg/dl)	
	Normal Value	Cold Climatic condition value
Humen (Homo Sapiense)	178.03 ± 32.02	162.01 ± 28.04^{++}
Goat (Capra Hircus)	105.01 ± 15.04	90.04± 11.06^{++}
Sheep (Ovis aries)	63.02 ± 5.07	45.06 ± 04.09^{++}
Cow (Bos Indicus)	82.06 ± 13.03	65.03 ± 12.05^{++}
Dog (Canis Familiaris)	172.04 ± 36.01	145.02 ± 25.03^{++}

Table No. 60 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

The data obtained in cold climatic have highly significant ($P < 0.01$) difference was observed in the cholesterol content between normal and cold climatic condition.

The mean value of cold climatic data were Humen Hemo sopien (162.01 ± 28.04) mg/dl., grat (Capra Hircus (90.04 ± 11.06) mg/dl. sheep ovis ovis (45.06 ± 04.09) mg/dl. cow - Bos indicus (65.03 ± 12.05) mg/dl., and Dog - Canis familearis (144.02 ± 25.03). The value obtained arranging in increasing order of serum cholesterol were -

Humen > Dog > Goat > Cow > Sheep.

The serum cholesterol level were observed in hot climatic condition of the mammals.

➢ Which was presented in (Table-61) The data obtained in hot climatic condition have highly significant ($P < 0.01$) difference was observed in the cholesterol content between normal and hot climatic condition.

The mean value of hot climatic data were.

Humen - Hemo sapcense (159.04 ± 27.06) mg/dl. Goat - Capra Hercus (87.06 ± 10.03) mg/dl., Sheep - ovis ovis (42.08 ± 04.04) mg/dl. Cow Bas indicus (62.05 ± 11.01) mg/dl. and Dog - canis familearis. (142.02 ± 23.02) mg/dl.

The value obtained arranging in increasing order of the serum cholesterol will be the some as in cold climatic condition & normal condition.

Humen > Dog > Goat > Cow > Sheep.

SERUM CHOLESTEROL LEVEL IN HOT CLIMATIC CONDITION OF THE MAMMALS

Mammalian (Species)	Serum cholesterol level (mg/dl)	
	Normal Value	Hot Climatic condition value
Humen (Homo Sapiense)	178.03 ± 32.02	159.04 ± 27.06^{++}
Goat (Capra Hircus)	105.01 ± 15.04	87.06 ± 10.03^{++}
Sheep (Ovis aries)	63.02 ± 5.07	42.08 ± 04.04^{++}
Cow (Bos Indicus)	82.06 ± 13.03	62.05 ± 11.01^{++}
Dog (Canis Familiaris)	172.04 ± 36.01	142.02 ± 23.02^{++}

Table No. 61 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

➢ The serum cholesterol level were also observed in different climatic condition (season) of the Experimented mammals (Human, Goat, Sheep, Cow and Dog). Which was presented in (Table-62).

The data obtained in different climatic condition (Season) have highly significant ($p < 0.01$) difference summer than winter.

All these correlation among the mammals suggest adaptive change in serum cholesterol level in response to changing physiological and environmental condition.

➢ The serum cholesterol level also observed in protein rich diet of the mammals.

Which was presented in (Table-63).

The data obtained in Protein rich diet after 60 days.

The does of protein rich diet were according to as per I.C.A.R. feeding standard (1985). Samples of feed and faeces were analysed for proximate. The composition and cell was constituents (A.O.A.C. 1980, Goering and van soest 1970).

The mean value of protein rich diet data were Human- Homo sapiense (189.06 ± 34.03) mg/dl., Goat- capar hircus (116.05 ± 17.07) mg/dl, Sheep ovis ovis (73.09 ± 9.05) mg/dl, Cow - Bos indicus (93.04 ± 15.04) Dog - Canis familiaris (183.3 ± 37.02) mg/dl. The mean value obtained, arranging in increasing order will be- Human > Dog > Goat > Cow > Sheep.

The serum cholesterol level were observed in lactating periods of the mammals which was presented in table. Which was presented in (Table-64).

SERUM CHOLESTEROL LEVEL IN DIFFERENT CLIMATIC CONDITION (SEASION) OF THE MAMMALS

Mammalian (Species)	Blood Glucose level in (Mg/dl)		
	Mansoon[1]	Winter[2]	Summer[3]
Humen (Homo Sapiense)	178.03 ± 32.02	162.01 ± 28.04++	159.04 ± 27.06+
Goat (Capra Hircus)	105.01 ± 15.04	90.04 ± 11.06++	87.06 ± 10.03+
Sheep (Ovis aries)	63.02 ± 5.07	45.06 ± 04.09++	42.08 ± 04.04+
Cow (Bos Indicus)	82.06 ± 13.03	65.03 ± 12.05++	62.05 ± 11.01+
Dog (Canis Familiaris)	172.04 ± 36.01	145.02 ± 25.03++	142.02 ± 23.02+

Table No. 62 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

SERUM CHOLESTEROL LEVEL IN PROTEIN RICH DIET OF THE MAMMALS

Mammalian Species	Serum cholesterol level (mg/dl)	
	Normal Value	Hot Climatic condition value
Humen (Homo Sapiense)	178.03 ± 32.02	189.06 ± 34.03
Goat (Capra Hircus)	105.01 ± 15.04	116.05 ± 17.07
Sheep (Ovis aries)	63.02 ± 5.07	73.09 ± 9.05
Cow (Bos Indicus)	82.06 ± 13.03	93.04 ±15.04
Dog (Canis Familiaris)	172.04 ± 36.01	183.03 ± 37.02

Table No. 63 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

SERUM CHOLESTEROL LEVEL IN LACTATING PERIOD OF THE MAMMALS

Lactating Days	Goat	Sheep	Cow
Day of Kidding	1050.3 ± 15.01	63.04 ± 5.02	82.04 ± 13.04
3rd day of lactating	115.08 ± 16.04	74.06 ± 7.03	93.02 ± 14.06
7th day of lactating	120 .04 ± 18.02	82.03 ± 3.08	98.03 ± 17.05
30th day of lactating	127 .01 ± 20.03	93.02 ± 6.07	110.06 ± 6.02
90th day of lactating	110 .06 ± 17.06	68.05 ± 4.04	88.08 ± 9.03

Table No. 64 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

The data obtained in lactating periods of the experimental mammals. (Goat, Sheep and Cow) have highly significant ($P < 0.05$) difference in serum in cholesterol level.

Cholesterol content was lower ($P < 0.01$) on the day of kidding which gradually increased till 30th day of lactation followed by fall on 90the post kidding. Since use of acetate in the milk fat synthesis would decrease after peak lactation, it would have contributed to higher cholesterol synthesis observed on 30th day of lactation.

The Activity of enzyme Alkaline Phosphates in different mammals. EC- 3.1.3.1 :

The activity of enzyme alkaline phosphate were observed in serum in different mammals.

Serum separated from the samples collected was stored in refrigerator until analysis were completed. Mostly fresh separate serum was used for analysis of enzyme activity.

The procedure was followed by kind and king (1954) and through kit mac comb R.B. et al. (1972). Expert panel of enzymes 1983.

The enzyme alkaline phasphatase activity were measured by colorimetically at 620 n m. And By kit chemistry auto analizer R.A. 50 used for reading.

➢ The activity of serum alkaline phasphalase obtained in normal condition of the mammals presented in (Table-65).

In Human Hemo sapiense (78.00 ± 0.21) IU/L, Goat Capra Hircus (68.70 ± 5.52) IU/h, Cow Bos indicus (36.50 ± 0.50) and Dog camis familearis (37.20 ± 081) IU/L.

SERUM ALKALINE PHASPHATAS ACTIVITY IN NORMAL CONDITION OF THE MAMMALS (E.C. 3.1. 3.1)

Mammalian (Species)	**Alkaline Phasphotase (IU/L)**
Humen (Homo Sapiense)	78.00 ± 0.21
Goat (Capra Hircus)	68.70 ± 5.52
Sheep (Ovis aries)	98.92 ± 12.08
Cow (Bos Indicus)	36.50 ± 0.50
Dog (Canis Familiaris)	37.20 ± 0.81

Table No. 65 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

King et al. (1954) reported that alkaline phasphatase activity in Human serum was 4-11 K. A.U. or 28 - 80 IU/L.

To convert K.A. unit into I.U/L multiply the result by 7.1.

The serum alkaline phasphatase activity were highly significant ($P < 0.05$) difference in each experimented memmals.

The serum alkaline phosphatase activity higher in sheep ovis ovis. Yet lowest in Cow (Bos indicus) If we arranging the mean value obtained in increasing order will be Sheep > Human > Goat > Dog > Cow.

Alkaline phosphatase is widely distributed enzyme but more plentiful in liver, bones, small intestine and placenta.

Out of studies in experimental mammals there are relatively abundant papers on the intestinal A.L.P. in mammals. rat (Hanna et al. 1979 Ydlin et al. 1981. Gilles-Baillien. 1984 Tardi vel et al. 1988. Unalkami et al., 1990). Mouse (Moog et al. 1966).

Human ALP shows differentiation into isozymes with tissue-specific distribution. intestinal placental, germ cell and tissue-unspecific ALPs (Moss, 1984a; Stigbrand, 1984b, Sussman. 1984. Harris, 1989: Fishman, 1990. Moss, 1992).

Despite the extensive studies on ALPs. their physiological roles are not well understood. The distribution of ALPs indicated that this enzyme is connected with absorption and transport mechanisms in several organs. and the close relationship of ALPs with Ca ATase. Mg^{2+} - ATPase and Na^{+}, K^{+} -ATPase was pointed out (Russell et al., 1972 Mc Comb et al. 1979 Brahmacupta and Melnykovych. 1980, Williams et al., 1985. Pizauro et al. 1993)

SERUM ALKALINE PHASPHATASE ACTIVITY IN PROTEIN RICH DIET OF THE MAMMALS (E.C. - 3.1.3.1.)

Mammalian Species	Alkaline Phasphotase Activity (IU/L)	
	Normal Values	Protein Treated Values
Humen (Homo Sapiense)	78.00 ± 0.21	88.08 ± 0.12^{++}
Goat (Capra Hircus)	68.70 ± 5.52	77.82 ± 4.98^{++}
Sheep (Ovis aries)	98.92 ± 12.08	109.01 ± 11.12^{++}
Cow (Bos Indicus)	36.50 ± 0.50	46.82 ± 0.18^{++}
Dog (Canis Familiaris)	37.20 ± 0.81	47.28 ± 0.82^{++}

Table No. 66 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

On the other hand the liver and bone ALPs have been utilized as valuable diagnostic tools to monitor hepatobiliary and bone diseases (Stigbrand. 1984: Moss. 1984b, 1992).

➢ The activity of enzyme alkaline phasphatase also observed in protein rich diet of the mammals.

Which was presented in (Table-66).

Significant ($P < 0.05$) an increase in alkaline pahsphatase activity was observed at 60 days of protein rich feeding in each experimented mammals (Human, Goat, Sheep, Cow & Dog) The does of protein rich diet were according to as per ICAR feeding standard of feed and faces were analysed for proximate composition and cell wall constetuent (AOAC 1980) Goering and Van soest 1970. The mean value of protein rich diet data were- Human Homo sapepinse (88.08 ± 0.12) IU/L, Goat capra hicus (77.82 ± 4.98) IU/L, Sheep- ovis ovis (109.01 ± 11.12) IU/L, Cow- Bos indicus (46.82 ± 0.18) IU/L, and Cow-Bos inducus (47.28 ± 0.82) IU/L.

According to mean values obtained arranging in increasing order will be same as normal condition of serum enzyme alkaline phaspate activity.

Sheep > Human > Goat > Dog > Cow.

Lower alkaline phaspate serum activity was the most striking event observed for this variable. Similar results were also observed by other in animals with food restriction (Healy and McInnes 1975; Lynch and Jackson 1983b; Cole 1992) and were probably caused by lower rats in bone metabolism and in phosphorylation and dephosphorylation reaction. Result also show that alkaline phaspate level was affected by undernutrition but not by overnutrition.

SERUM ALKALINE PHASPHATASE ACTIVITY DURING PREGNANCY OF THE MAMMALS (E.C. 3.1.3.1)

Mammalian Species	Day of Matting	Alkaline Phasphatase Activity (IU/L) Pregnency (days)			
		30th days	60th days	90th days	120th days
Goat (Capra Hircus)	68.80 ± 5.20	77.60 ± 4.40++	82.65 ± 5.35+	91.13 ± 6.07+	98.90 ± 6.10+
Sheep (Ovis aries)	98.92 ± 12.08	105.82 ± 13.12++	111.21 ± 12.32+	120.13 ± 12.43+	126.14 ± 12.52+
Cow (Bos Indicus)	36.50 ± 1.50	46.16 ± 1.30++	51.23 ± 1.87+	60.31 ± 1.92+	65.42 ± 1.94+

Table No. 67 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

In conclusion, a daily profile was observed in almost al variables, which may be of importance when interpretin these blood indicators of metabolic state. In order to maximise the diagonstic value of those indicators, the meal allows the characterisation of a relaviely steady metabolic state, intermediate between the effects of food intake and the final phase of the intensification of body reserve mobilisation.

The activity of enzyme alkaline phaspetase also observed in during pregnency of the experimental mammals (Goat, Sheep & Cow) which is presented in (Table-67).

The mean values of alkaline phasphatase activity increased linearly from the day of matin to during 120 day pregnency in each case of the mammals.

The difference being significantly from second month of pregnency the increased level of alkaline phasphatase during pregnency was simlar as reported by Healy (1971) Sharma and Ray (1985).

The increase in enzyme activity appear to be due to the liberation of enzyme form placenta during pregnency Mehta et al. (1989) expressed that higher phasphates are required for implantation.

- The activity of enzyme alkaline phasphatase also observed in Parturition and after parturition in experimented mammals (Goat Sheep and Cow). Which was presented in (Table-68).

The mean value of alkaline phasphatase activity highly significant ($P < 0.01$) difference from normal condition to day of parturition and after 7th days parturition. The activity of enzyme

SERUM ALKALINE PHASPHATASE ACTIVITY IN PARTURITION AND AFTER PARTURITION OF THE MAMMALS

Mammalian (Species)	Alkaline Phasphatase activity (IU/L)		
	Normal Value	**Parturation day Value**	**7th day after Parturation value**
Goat (Capra Hircus)	68.80 ± 5.20	109.27 ± 6.33++	107.50 ± 4.65+
Sheep (Ovis aries)	98.92 ± 12.08	132.83 ± 12.18++	129.71 ± 12.27+
Cow (Bos Indicus)	36.50 ± 1.50	69.83 ± 1.64++	66.72 ±1.73+

Table No. 68 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

alkaline phaspatase leneraly increase from the day of matting to day of parturition.

The increase in enzyme activity appear to be due to the libration of enzyme of placenta during pregnency till parturition expressed that higher phasphate are required for implantation.

After parturition there were no need of phasphate to placenta so after 7the days of parturition enzyme alkaline phasphate activity automatically decreased in each case of mammals.

The activity of enzyme lactate dehydrogenase in different mammals (E.C. - 1.1.1.27) :

The activity of enzyme lactate dehydrogenase were observed in serum of different mamals.

Serum separated from the samples collected was stroed in refrigerator until analysis were completed. Mostly fresh separated serum was used for anallyssi of enzyme activity.

The procedure was followed by Cabaud P et. al 1958 and by kit through analizer searey R.L. et. al (1969) and Lum G. et. al (1974). The enzyme Lactatedhy drogenase activity were measured by colori metrically at 540 nm. and through ket chemistry outoanalizm RA - 50 used for reading.

The activity of serum Lactate dehydrogenase obtained in normal condition of the mammals. Presented in (Table-69) In humen Homo sapiense (144.08 ± 18.12 IU/L, Goat - Capra Hircus (271.42 ± 27.93) Sheep ovis ovis (373.21 ± 19.39) IU/L. Cow - Bos indicus (291.34 ± 24.28) IU/L. & Dog canis familiaris (168.25 ± 21.23) IU/H.

SERUM LACTATE DEHYDROGENASE ACTIVITY IN NORMAL CONDITION OF THE MAMMALS (E.C. - 1.1.1.27)

Mammalian (Species)	**Serum lactate dehydrogenase activity (IU/L)**
Humen (Homo Sapiense)	144.08 ± 18.12
Goat (Capra Hircus)	271.42 ± 27.93
Sheep (Ovis aries)	373.21 ± 19.39
Cow (Bos Indicus)	291.34± 24.28
Dog (Canis Familiaris)	168.25 ± 21.23

Table No. 69 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

Cabaurd et al (1958) reported that Lactate dehydrogenase activity in humen 50-170 IU/L.

The serum Lactate dehydrogenises activity were higher in sheep where as lowest in humen. It we arranging the mean value obtained in increasing order will be sheep > cow > Goat > Dog > Humen.

➢ The enzyme serum Lactatedehydro genase activity also observed in grazing mammals.

The data was presented in (Table-70)

The data obtained in grazing mammals data were Goat (302.08 ± 23.02) IU/L, Sheep (405.28 ± 21.32) IU/L and in Cow (322.0 ± 18.32) IU/L.

The serum enzyme Lactate dehyderogenase activity obtained arranging in increasing order will be - sheep > cow > Goat. The serum enzyme Lactatedehy drogenase activity highly. increase in grazing mammals.

When mammals graze on wheat pusture or old cereal grain they develop a condition kwnon as wheat pasture poisoning Bohaman et. al (1983b.) Sympton like ataxia steffness staggering and falling profuse solivation are seen in it (Blood et al 1983), Due and Nauriyal 1988.

➢ The activity of enzyme lactate dehydrogenase also observed in during pregnancy of the mammals whichwas presented in (Table-71)

The mean value of enzyme lactate dehydrogenase increased llinarly from the day of mating to the 120th day of pregnancy.

SERUM LACTATE DEHYDROGENASE ACTIVITY IN GRAZING MAMMALS (E.C. - 1.1.1.27)

Mammalian (Species)	Serum Lactate dehydrogenase activity (IU/L)	
	Normal Values	**Grazing mammals**
Goat (Capra Hircus)	271.42 ± 27.93	302.08 ± 23.02^{++}
Sheep (Ovis aries)	373.21 ± 19.39	405.28 ± 21.32^{++}
Cow (Bos Indicus)	291.34 ± 24.28	322.07 ± 18.32^{++}

Table No. 70 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

SERUM LACTATE DEHYDROGENASE ACTIVITY DURING PREGNANCY OF THE MAMMALS (E.C. - 1.1.1.27)

Mammalia Species	Day of Matting	Serum lactate dehydrogenase activity (IU/L)			
		30th days	60th days	90th days	120th days
Goat (Capra Hircus)	271.42 ± 27.93	276.00 ± 24.46+	278.08 ± 22.32+	284.66 ± 18.46+	297.50 ± 28.52+
Sheep (Ovis ovis)	373.21 ± 19.39	377.38 ± 18.08+	380.29 ± 12.08+	384.23 ± 14.18+	39412 ± 13.16+
Cow (Bos Indicus)	291.34 ± 24.28	297.52 ± 21.18+	299.06 ± 14.42+	305.12 ± 15.16+	316.34 ± 14.36+

Table No. 71 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

The different being significantly difference from 3rd month of pregnency.

The increase in LDH activity during pregnency similar reported by Sharma and Roy in goats and Prabhaker et. al. (1999) in buffoloes.

➢ The activity of enzyme lactate dely drogenase observed in Parturetian and after parturition in experimented mammals (Goat Sheep & Cow). Which was presented in (Table-72).

The mean value of located dehy drogenase activity highly significant ($P < 0.01$) difference from normal condition to dya of parturitien and 7th days of parturition. The activity of enzyme lactate deley drogenase linearly increase from the day of mating to day of parturition.

The increase in enzyme. Bassler and Breth feld (1968) reported higher loctate dely drogenose activity in mammary parenchyma during iniiation of lactation.

Growth of foetus during gestatien and initetation of lactation on the day of parturition might be resposnsibel for increased lactate dely drogenase activity.

The activity of Serum glutamic oxalo acetic transaminase (SGOT) in different mammals.

The activity of enzyme (SGOT) were observed in serum of different mammals. Serum separated from the sample collected was stored in refrigerator until analysis were completed mostly fresh reprted serum was used form amalysis of enzyme (SGOT) activity.

SERUM LACTATE DEHYDROGENASE ACTIVITY DAY OF PARTURITION AND AFTER PARTURITION OF THE MAMMALS (E.C. - 1.1.1.27)

Mammalian (Species)	Serum Lactate dehydrogenase activity (IU/L)		
	Normal Value	**Parturition day Value**	**7th day after Parturition value**
Goat (Capra Hircus)	271.42 ± 27.93	$308.17 \pm 32.63^{++}$	$316.52 \pm 27.48^{+}$
Sheep (Ovis ovis)	373.21 ± 19.39	$405.24 \pm 19.36^{++}$	$412.16 \pm 18.34^{+}$
Cow (Bos Indicus)	297.52 ± 21.18	$332.19 \pm 21.23^{++}$	$339.18 \pm 21.32^{+}$

Table No. 72 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

The procedure was follwed by reitman and frankel (1957) and by kit expert panel of the IFCC on enzyme (1976).

The enzyme (SGOT) Activity were measured by colorimetrically at 540 nm. and by kit through chemistry auto analizer RA-50 use for reading.

➢ The activity of SGOT obtained in normal condition of the mammals presented in (Table-73).

In humen Homo sapiense (17.23 ± 2.08) IU/L, Goat Capra Hircus (70.26 ± 1.34) IU/L, sheep ovis ovis (87.95 ± 2,31) IU/L. Cow - Bos indicus (42.32 ± 1.83) IU/L & in Dog - conis fomiliaris (21.12 ± 1.77) IU/L & in Dog - coins familiaris (21.32 ± 1.77) IU/L. Reitman et al (1957) reported SGOT activity mean of range in humen. 2-20 IU/L. The serum (GOT) activity highly significant in each experimental mammals.

The serum GOT activity was higher in sheep and lowest in humen.

If we arranging the mean values obtained in increasing order will be sheep > Goat > Cow > Dog > Human.

Normal values have been reported in heart conditions without infarction, such as angina and pericarditis, in patients with pulmonary embolism and also in patients with acute abdominal attacks. Foulk and Fleisher (1959) found an increase in over 50 percent patients with acuute pancreatitis.

The enzyme is abundant, widely distributed physiologically important and is the best characterized of the transaminases. It has been purified extensively from pig heart by combined heat-

SERUM GLUTAMIC OXALOACETIC TRANSAMINASE (SGOT) ACTIVITY IN NORMAL CONDITION OF THE MAMMALS

Mammalian (Species)	(SGOT) Activity (IU/L)
Humen (Homo Sapiense)	17.23 ± 2.08
Goat (Capra Hircus)	70.26 ± 1.34
Sheep (Ovis ovis)	87.95 ± 2.31
Cow (Bos Indicus)	42.32 ± 1.83
Dog (Canis Familiaris)	21.13 ± 1.77

Table No. 73 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

denaturation of impurities, and occurs in two forms which are interconvertible by the addition of excess glutamate or α–κetoglutarate. Acid or alkaline hydrolysis splits from the enzyme either pyriodoxal phosphate or pyridoxamine phosphate.

➢ The activity of enzyme SGOT. Also observed in protein rich diet of the mammals.

Which was presented in (Table-74).

Significance (P > 0.05) difference decrease the SGOT activity observed at 60 days of protein rich feeding in each experimented mammals (Humen Homo sopiense, Goat - Capra Hircus) sheep ovis ovis, Cow - Bos indicus & Dog - canis familearis). The does of protein rich diet were according to as per ICAR feeding standard (1985).

Sample of feed and faeces were analysed for proximate composition and cell wasll constituent (AOAC 1980) Goring and Vas Soest 1970). The mean value of protein rich diet 60th day data a were Humen (17.13 ± 2.18) IU/L, Goat (69.32 ± 1.38) IU/L. Sheep (86.83 ± 2.41) IU/L, Cow (41.42 ± 1.84) IU/L. & Dog (20.14 ± 1.76) IU/L.

According to mean values obtained arranging in increasing order will be sheep > Goat > Cow > Dog > Humen.

➢ The activity of enzyme SGOT also observed in starvation period of the mammals which was presented in (Table-75).

The data obtained in 24 hours starvation period have highly significant (P > 0.01) difference increase was observed in SGOT activity in starvation them normal condition of the mammals in each cases.

SERUM GLUTAMIC OXALOACETIC TRANSAMINASE (SGOT) ACTIVITY IN PROTEIN RICH DIET OF THE MAMMALS

Mammalian (Species)	(SGOT) Activity in Protein rich diet (IU/L)	
	Normal Value	**After protein rich diet value**
Humen (Homo Sapiense)	17.23 ± 2.08	17.13 ± 2.18
Goat (Capra Hircus)	70.26 ± 1.34	69.32 ± 1.38
Sheep (Ovis ovis)	87.95 ± 2.31	86.83 ± 2.41
Cow (Bos Indicus)	42.32 ± 1.83	41.42 ± 1.84
Dog (Canis Familiaris)	21.13 ± 1.77	20.14 ± 1.76

Table No. 74 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

The mean value of starvation data were Humen - Homo suiencse (23.24 ± 2.18) IU/L, Goat - Capra Hircus (75.27 ± 1.35) IU/L. Sheep Ovis Ovis (91.85 ± 2.41) IU/L, Cow - Bos indicus (49.43 ± 185) IU/L & Dog Conis Familiari (28.21 ± 1.78) IU/L.

The obtained arraigning in increasing order of SGOT activit were -

The transaminase activity is entimately related with the protein cetabolism and subsequet production of keto acid. The Keto acid can further be utilized for glucoeogenesis 6 smith and Hoizer (1967).

Lesser utilization of glucose and content glucose neogenesis during starvation maybe responsible for maintaining blood glucose at basal level in mammals.

The activity of Serum Glutamic pyruvic transaminase (SGPT) in different mammals :

The activity of enzyme (SGPT) were observed in serum of different mammals. Serum separated from the sample collected was stored in refrigerator until analysis were completed mostly fresh reprted serum was used form amalysis of enzyme (SGPT) activity. The procedure was follwed by reitman and frankel (1957) and by kit expert panel of the IFCC on enzyme (1976).

The enzyme (SGPT) Activity were measured by colorimetrically at 540 nm. and by kit through chemistry auto analizer RA-50 use for reading.

➢ The activity of SGPT obtained in n romal condition of the mammals presented in (Table-76).

SERUM GLUTAMIC OXALOACETIC TRANSAMINASE (SGOT) ACTIVITY IN STARVATION OF THE MAMMALS

Mammalian (Species)	(SGOT) Activity (IU/L)	
	Normal Value	**Starvation after 24 hrs. mammals**
Humen (Homo Sapiense)	17.23 ± 2.08	23.24 ± 2.18+
Goat (Capra Hircus)	70.26 ± 1.34	75.27± 1.35+
Sheep (Ovis ovis)	87.95 ± 2.31	91.85 ± 2.41+
Cow (Bos Indicus)	42.32 ± 1.83	49.43 ± 1.85+
Dog (Canis Familiaris)	21.13 ± 1.77	28.21 ± 1.78+

Table No. 75 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

SERUM GLUTAMIC PYRUVIC TRANSAMINASE (SGPT) ACTIVITY IN NORMAL CONDITION OF THE MAMMALS

Mammalian (Species)	(SGPT) Activity (IU/L)
Humen (Homo Sapiense)	14.02 ± 1.03
Goat (Capra Hircus)	29.45 ± 1.35
Sheep (Ovis ovis)	45.12 ± 1.83
Cow (Bos Indicus)	17.76 ± 1.34
Dog (Canis Familiaris)	22.50 ± 1.20

Table No. 76 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

In Humen homo sapiense (4.02 ± 1.03) IU/L, Goat (Capra hircus) (29.45 ± 1.35) IU/L, Sheep (Ovis Ovis) (45.12 ± 1.83) IU/L, Cow (Bos Indicus) (17.76 ± 1.34) IU/L and Dog (Canis Familiaris) (22.50 ± 1.20) IU/L.

Reitman et. al (1957), reported SGPT activity mean of range in Humen 2 - 15 IU/L. If we arranging the mean value obtained in increasing order will be sheep > Goat > Cow > Dog > Humen.

SGPT is not usually elevated in myocardial infraction unless the lesion is a large one or there is associated liver damage.

Both the enzymes show elevated levels in hepatocellular damage e.g. due to hepatotoxic drug, infective hepatitis and primary or secondary liver cancer. Raised levels are also seen in Laennec's cirrhosis, biliary cirrhosis and obstructive jaundice. In infective hepatitis the increase is particularly marked and highest values are obtained. In early stage SGPT is more increased than SGPT, but SGPT exceeds SGPT in advanced stage. In outbreaks of infective hepatitis estimation of serum transaminases is the most sensitive diagnostic index. Increase begins in the prodromal period and the determination can be of great value. An activity in the normal range would appear to exclude this condition.

➢ The activity of enzyme SGPT. Also observed in protein rich diet of the mammals. Which was presented in (Table-77).

Significance significant (P > 0.05) difference decrease the SGPT activity observed at 60 days of protein rich feeding in each experimented mammals (Humen Homo sopiense, Goat - Capra Hircus) sheep ovis ovis, Cow - Bos indicus & Dog - canis familearis).

SERUM GLUTAMIC PYRUVIC TRANSAMINASE (SGPT) ACTIVITY IN PROTEIN RICH DIET OF THE MAMMALS

Mammalian (Species)	(SGPT) Activity (IU/L)	
	Normal Value	**Protein rich diet value**
Humen (Homo Sapiense)	14.02 ± 1.03	14.13 ± 1.13
Goat (Capra Hircus)	29.45 ± 1.35	29.56 ± 1.45
Sheep (Ovis ovis)	45.12 ± 1.83	45.13 ± 1.93
Cow (Bos Indicus)	17.76 ± 1.34	17.76 ± 1.38
Dog (Canis Familiaris)	22.50 ± 1.20	22.82 ± 1.28

Table No. 77 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

SERUM GLUTAMIC PYRUVIC TRANSAMINASE (SGPT) ACTIVITY IN STARVATION OF THE MAMMALS

Mammalian (Species)	(SGPT) Activity (IU/L)	
	Normal Value	**Starvation after 24 hrs. mammals value**
Humen (Homo Sapiense)	14.02 ± 1.03	25.12 ± 1.13^{++}
Goat (Capra Hircus)	29.45 ± 1.35	41.46 ± 1.45^{++}
Sheep (Ovis ovis)	45.12 ± 1.83	57.13 ± 1.87^{++}
Cow (Bos Indicus)	17.76 ± 1.34	29.64 ± 1.36^{++}
Dog (Canis Familiaris)	22.50 ± 1.20	33.60 ± 1.32^{++}

Table No. 78 : ± Standard Deviation

Each value is the mean of at least 10 -12 experiments.

+ $P < 0.05$ (Significant difference)

++ $P < 0.01$ (Significant difference)

The does of protein rich diet were according to as per ICAR feeding standard (1985).

Sample of feed and faeces were analysed for proximate composition and cell wasll constituent (AOAC 1980) Goring and Vas Soest 1970).

The mean value of protein rich diet 60th days data were -

Humen (Homo Sapiense) 14.13 ± 1.13 IU/L, Goat (Capra Hircus) 29.56 ± 1.45 IU/L, Sheep (Ovis Ovis) 45.13 ± 1.93 IU/L, Cow (Bos Indicus) 17.76 ± 1.38 IU/L & Dog (Canis Familiaris) 22.82 ± 1.28 IU/L.

According to mean values obtained arranging in increasing order will be sheep > goat > cow > dog > humen.

➢ The activity of enzyme SGOT also observed in starvation period of the mammals which was presented in (Table-78).

The data obtained in 24 hours starvation period have highly significant (P > 0.01) difference increase was observed in SGOT activity in starvation them normal condition of the mammals in each cases.

The mean value of starvation data were Humen - Homo suiencse (Homo Sapiense) 25.12 ± 1.13 IU/L, Goat (Capra Hircus) 41.46 ± 1.45 IU/L, Sheep (Ovis Ovis) 57.13 ± 1.87 IU/L, Cow (Bos Indicus) 29.64 ± 1.36 IU/L & Dog (Canis Familiaris) 33.60 ± 1.32 IU/L.

The obtained arraigning in increasing order of SGOT activit were -

The transaminase activity is entimately related with the protein cetabolism and subsequet production of keto acid. The Keto acid can further be utilized for glucoeogenesis 6 smith and Hoizer (1967).

Lesser utilization of glucose and content glucose neogenesis during starvation maybe responsible for maintaining blood glucose at basal level in mammals.

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